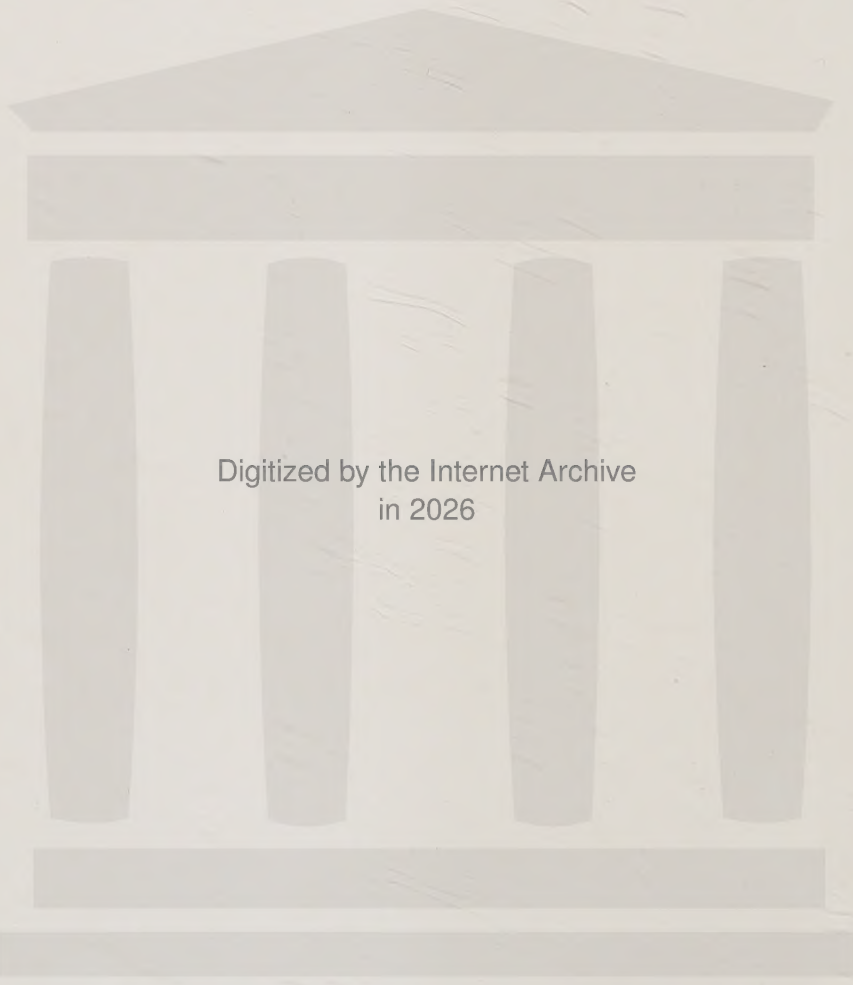


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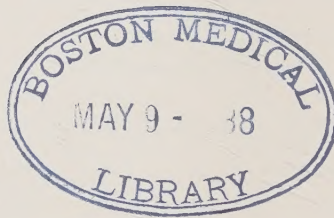
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THE RELATION OF THE SCIATIC NERVE AND OF ITS SUBDIVISIONS TO THE PIRIFORMIS MUSCLE

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ONE PLATE

The various relationships which may exist between the sciatic nerve and the piriformis muscle are described in textbooks of anatomy. The percentage incidence of variation from the 'normal' and of the different types of variation are, however, presented in but few. In the present study of 120 consecutive cases from student dissections, the types were recorded and illustrations prepared from actual specimens (figs. a to e).

The essential anatomy of the relation between the sciatic nerve and the piriformis muscle may be briefly described. Usually the nerve emerges from the pelvis by passing beneath the piriformis muscle. The muscle, however, may split into two quite separate heads; the nerve too may divide, within the pelvis, into its tibial and common peroneal portions. Thus six combinations are possible: these are tabulated herewith (table 1). Only four (figs. a to d) of the six types of arrangement were observed in our specimens; the percentage occurrence of each of the four is recorded in the following table, to which is added the percentages offered by Parsons and Keith (1896).

¹Contribution no. 254 from the Anatomical Laboratory of Northwestern University Medical School.

TABLE 1

	BEATON AND ANSON	PARSONS AND KEITH
		%
Undivided nerve below undivided muscle	84.2% (fig. a)	85
Divisions of nerve between and below undivided muscle	11.7% (fig. b)	12.3
Divisions above and below undivided muscle	3.3% (fig. e)	
Undivided nerve between heads	0.8% (fig. d) ¹	2.2
Divisions between and above heads	None (fig. f)	
Undivided nerve above undivided muscle	None (fig. g)	

¹ In an earlier case (fig. e), which is not included in the present percentages, the piriformis muscle, instead of dividing into two portions, the superior one of which overlapped the inferior but slightly, the superior member covered three-fourths of the latter's anterior surface. The nerve divided but not as in figures b and c; the part passing on the pelvic surface of the superior head was the fourth sacral component of the lumbosacral trunk, which, distal to the muscle-belly, joined the fifth lumbar and the first to third sacral nerves to form the sciatic nerve. Except for the relation of this fourth sacral segment, the sciatic passed in the interval between the two heads, being fundamentally similar, therefore, to the specimen in figure d.

Parsons and Keith alone record the incidence of the types of variation;² other investigators, however, present statistics on the total percentage of anomalous cases (compare, 'normal,' fig. a). Their results and our own may be shown by tabulation (table 2).

TABLE 2

INVESTIGATOR	DATE	PER CENT VARIATION	NATIONALITY
Eisler	1892	18	German
Paterson	1894	13	English
LeDouble	1897	17	French
Bardeen, Elting	1901	10	American
Trotter	1932	14	American (Whites and Negroes)
Berkol, et al.	1935	2.6	Turk
Fukumoto	1935	34.6	Japanese
Parsons, Keith	1896	15	English
Anson, Beaton	1937	15.8	American (Whites and Negroes)

Of our nineteen cases (15.8%) of variations five occurred unilaterally. The other fourteen cases were bilateral (i.e., on both sides of seven bodies). Of the five unilateral, two were

² Because of the uneven spread as to sex, no attempt is made to analyze results for sex differences. This has been done by Trotter, Berkol et al., Parsons and Keith, and Fukumoto, who also consider racial differences.

of the right side, three of the left. Parsons and Keith (1896) and Trotter ('32) found that unilateral variations were slightly more frequent on the left; Fukumoto ('35) and Berkol et al. ('35) record a greater incidence on the right side. In Trotter's series the incidence of bilateral and unilateral variation was approximately equal.

LITERATURE CITED

- BARDEEN, C. R., AND A. W. ELTING 1901 A statistical study of the variations in the formation and position of lumbo-sacral plexus in man. II. *Anatomische Anzeiger*, Bd. 19, S. 209-239.
- BERKOL, N., A. MOUCHET AND H. GÖGEN 1935 Note sur le niveau de bifurcation du grand nerf sciatique. *Annales d'Anatomie Pathologique et d'Anatomie Normale Medico-chirurgicale*, T. 12, pp. 596-600.
- EISLER, P. 1892 *Der Plexus lumbo-sacralis des Menschen*. Halle.
- FUKUMOTO, N. 1935 Über den M. piriformis und N. ischiadicus von Japanern. *Fukuoka Acta Medica*, vol. 28, p. 39.
- LEDOUBLE, A. B. 1897 *Traité des variations du système musculaire de l'homme*. T. 2. Paris.
- PARSONS, F. G., AND SIR A. KEITH 1896-1897 Sixth Annual Report of the Committee of Collective Investigation of the Anatomical Society of Great Britain and Ireland. First part. *J. Anat. and Physiol.*, vol. 31, pp. 31-44.
- PATERSON, A. M. 1894 The origin and distribution of the nerves of the lower limbs. *J. Anat. and Physiol.*, vol. 28, pp. 84-95.
- TROTTER, M. 1932 The relation of the sciatic nerve to the piriformis muscle in American Whites and Negroes. *Anat. Rec.*, vol. 52, pp. 321-323.

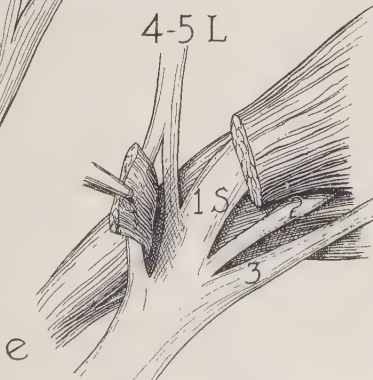
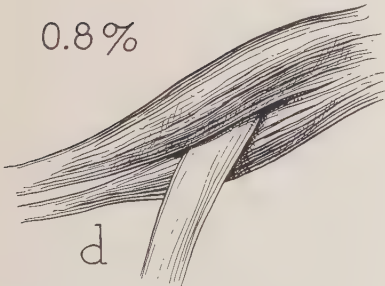
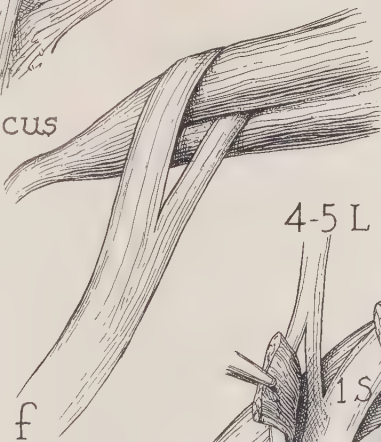
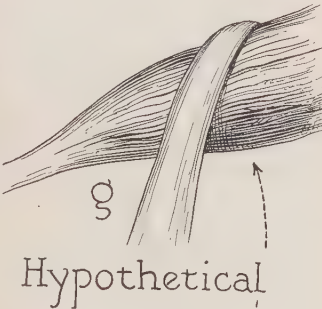
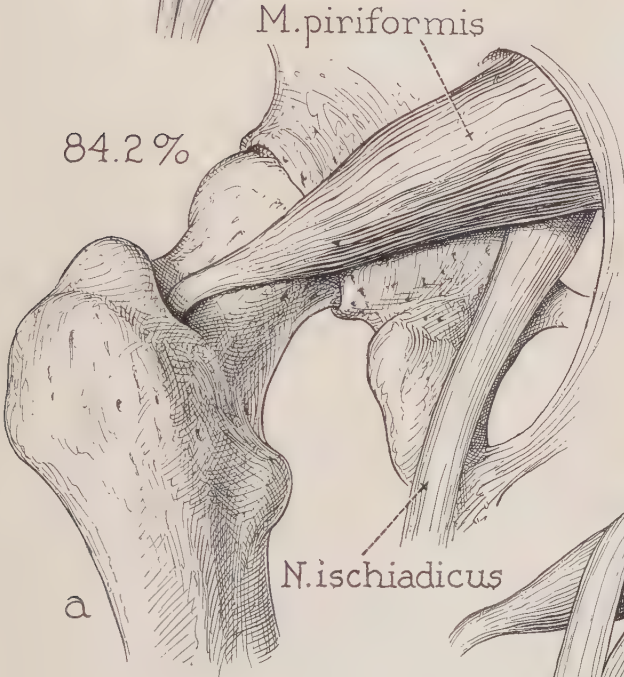
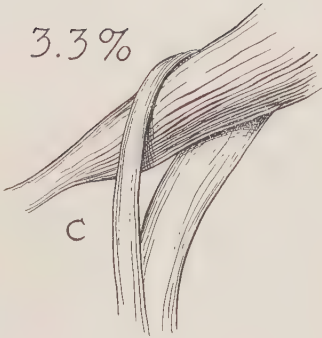
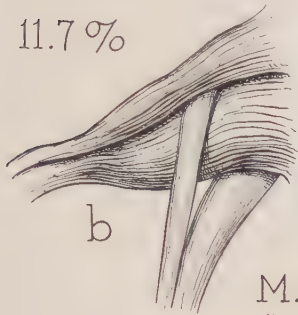
PLATE 1

EXPLANATION OF FIGURES

Figures a to g. Six types of arrangement of the sciatic nerve, or if its subdivisions, in relation to the piriformis muscle. Arranged in the order of frequency; the percentage incidence in 120 examples is indicated. Figures f and g, hypothetical; others are actual cases. Figure e, pelvic view, others gluteal views.

- a Nerve undivided passes out of the greater ischiadic foramen, below the piriformis muscle.
- b The divisions of the nerve pass between and below the heads of the muscle.
- c Divisions above and below the undivided muscle.
- d Nerve undivided between the heads of the muscle.
- e A variation of the preceding (see footnote 1, table 1).
- f Divisions of the nerve between and above the heads (hypothetical).
- g Undivided nerve above the undivided muscle (hypothetical).

Relation of
sciatic nerve
to piriformis
muscle



THE EFFECTS OF JARRING UPON THE EMBRY- OGENY OF CHICK EMBRYOS

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TWO FIGURES

A class of students in microtechnic opened some eggs which were incubated for sectioning, and discovered that the embryos were not normal. In these embryos the vitelline circulation did not present the anastomosing effect which is so characteristic of the early developmental stages; and closer examination showed the blood cells concentrated near the periphery of the blastoderm giving the impression of extravasated blood. Such a condition existed not only in one or two of the embryos examined, but in several of them; thus making it apparent that something had seriously disturbed the normal course of development. An effort was made to discover the cause of these embryonal disturbances. A few small fragments of rock were found near the incubator, and it was suspected that geology students had broken up rocks during the incubation period, and that the jarring thus produced affected the eggs. To verify this suspicion an investigation was made to determine whether such conditions as were found in the embryos could be produced by jarring and to discover what other effects such jarring might have.

METHOD

Eggs were subjected to jarring during various stages of development. The incubator in which the eggs were kept was placed on a table which had no contact with the walls of the laboratory, and jarring was produced by allowing a weight of 490 gm. to strike the table from a height of approximately

6 cm. On the average fifty-two blows were struck per minute at 15-minute intervals. Such jarring was sufficiently strong to produce perceptible movements of the egg, but was not severe enough to cause the air cells to migrate from their normal positions, a condition spoken of as tremulous air cells. A study by Knox and Olsen ('36) of the effects of tremulous air cells on hatchability indicated that eggs having normal air cells hatched much better than those with tremulous air cells.

Toto mounts were prepared of the various stages of the developing chick, namely 33-hour, 48-hour and 72-hour stages, and serial sections of 48-hour embryos were also prepared.

OBSERVATIONS AND EXPERIMENTAL RESULTS

The death rate of embryos submitted to jarring was extremely high. Only one egg hatched out of a total of 155 incubated. The percentage of hatchability was only 0.65%, as contrasted with a hatchability of 45% in the control. Roughly, 60% of the embryos died some time between the second and third days of incubation, and the others ceased to develop at various stages up to approximately 11 days of incubation.

Jarring for a 1-minute duration at 15-minute intervals from the fourth to the twelfth hours of incubation gave abnormal embryos. Two areas in particular appeared to be especially sensitive—the vitelline circulation, and various parts of the nervous system. When a 48-hour embryo was examined following jarring from the fourth to the twelfth hours of incubation, the first peculiarity observed was the absence of the extensive network of vitelline arteries and veins. In its development the vitelline circulation progressed, at least in part, no further than the blood island stage. Instead of freely-moving blood cells, the corpuscles were trapped in the blood islands. Sometimes these islands were spherical in shape; sometimes two, three or more were fused together, forming various-shaped structures; fre-

quently they were arranged in rows, giving evidence of the direction the blood vessels might have taken had their formation continued without interruption. The blood islands appeared swollen and distended with plasma. The blood was



Fig. 1 This represents an embryo incubated for 72 hours. Normal vitelline circulation is completely lacking. The blastoderm contains a large number of much enlarged blood islands, with the blood cells aggregated near the center of each island. The embryo proper is very much reduced in size, but contains many of the essential structures.

not extravasated, but was completely surrounded with endothelium. Under such conditions vitelline circulation was impossible. There was no connection between the blood islands, and no means whereby circulation could take place.

In its early developmental stages, the embryo is dependent upon the vitelline circulation for its oxygen supply, as well as for its nutriment. Therefore, the assumption is made that if the vitelline circulation fails to develop normally the embryo can live only until it becomes too large to receive sufficient food and oxygen by direct absorption through its surface. The vitelline circulation was not always completely

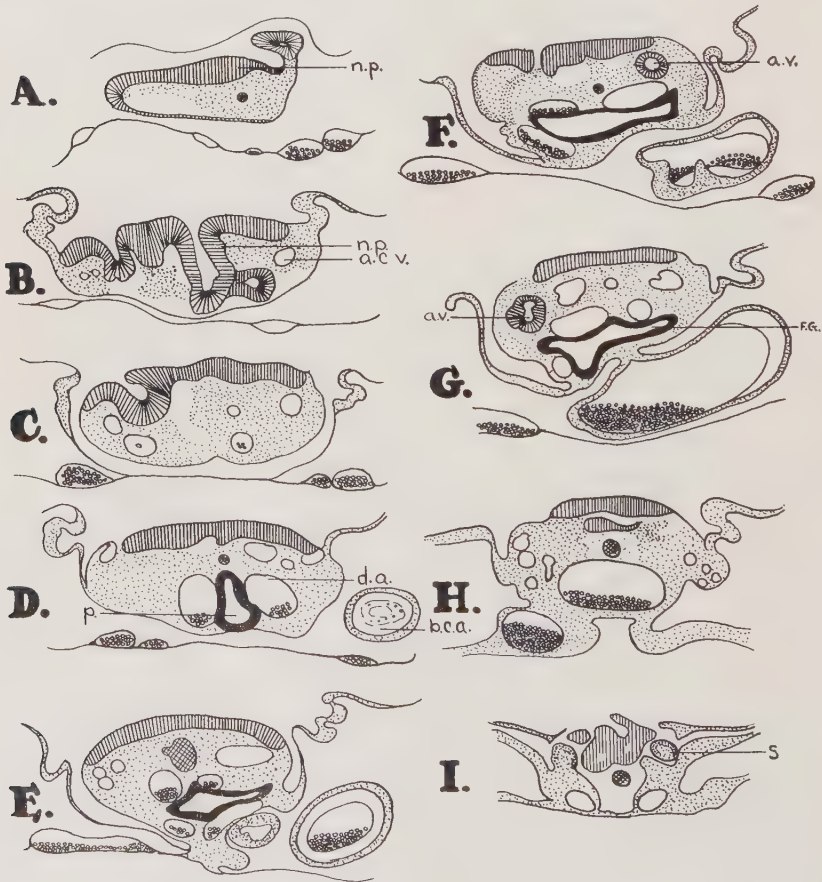


Fig. 2 Serial sections of a 48-hour chick, at intervals of 200 μ . In this specimen the nervous system has almost completely failed to develop. Auditory vesicles are present, but there is no indication of optic vesicles. n.p., neural plate; a.c.v., anterior cardinal vein; b.c.a., bulbo-conus arteriosus; p., pharynx; d.a., dorsal aorta; a.v., auditory vesicle; f.g., fore-gut; s., somite.

abnormal. In some instances one-half of the circulation developed quite normally, whereas the other half failed to do so. Sometimes only small areas of the yolk sac contained abnormal blood vessels. Perhaps this explains why a few of the embryos lived longer, but only to the point where their partly disorganized circulation was no longer capable of keeping them alive. Of the embryos proper not all were appreciably atypical, but of those which were, the aberrancy was extreme (fig. 1).

A study of the serial sections revealed several interesting facts. First of all, the heart and blood vessels within the embryo proper usually developed normally. The only perceptible variation was the small number of blood cells within the embryo. Second, certain characteristic anomalies of the nervous system became apparent. The most common of these were as follows: failure of the neural tube to form either in whole or in part. Sometimes the neural plate remained flat with no indication of neural groove formation throughout the entire length, but the other organs apparently were developing normally. Frequently, either the optic or auditory vesicles, or both were lacking (fig. 2).

An attempt was made to cultivate the chick embryo in an open egg according to methods employed by Romanoff ('31) and Byerly ('26), but with no success. Jarring caused the yolk sac to adhere closely to the shell membranes, with the result that the yolk was not free to rotate and the embryo failed to lie near the opening. If the eggs were opened prior to jarring, they failed to develop to any extent. It would be desirable to observe the embryos while they were developing in order to obtain information with respect to the time at which the heart ceased to beat, and in order to study the nature of such circulation as may have been established.

SUMMARY

1. Jarring the egg during incubation does exert deleterious effects upon the developing embryo.

2. When jarred for 1 minute duration at 15-minute intervals from the fourth to the twelfth hours of incubation, the vitelline circulation and various parts of the nervous system are the parts most frequently found to be teratological.

3. Jarring, such as that described above, reduces hatchability to approximately zero, 0.65%, to be exact.

4. Various insignificant abnormalities occur, consisting principally of localized cellular proliferations or additional blood vessels.

LITERATURE CITED

- BYERLY, T. C. 1926 A simple method for observation of the living chick embryo. *Science*, vol. 63, pp. 458-459.
- KNOX, CHARLES W., AND MARLOW W. OLSEN 1936 The effect of termulous air cells upon the hatchability of eggs. *Poultry Science*, vol. 15, pp. 345-348.
- ROMANOFF, ALEXIS L. 1931 Cultivation of chick embryos in an open egg. *Anat. Rec.*, vol. 48, pp. 185-189.

CYTOLOGICAL ABNORMALITIES IN THE OOCYTES OF THE 3-WEEK KITTEN'S OVARY

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THREE TEXT FIGURES AND THREE PLATES (EIGHTEEN FIGURES)

In studying some histological sections of a 3-week kitten's ovary, a tripolar metaphase was observed in one of the oocytes. Further examination of the same kind of material revealed several other types of cytological abnormalities. Since similar abnormalities in other tissues are known to lead to cellular degeneration, the idea is suggested that these irregular mitoses in the cat might take part in the degeneration which, according to von Winiwarter and Sainmont ('09), affects all of the primitive cortical cords of the ovary. If this were true, it would be of considerable importance in the general study of mammalian ovarian development, for the pioneer work on mammalian oogenesis was done by von Winiwarter and Sainmont ('00, '09) on the rabbit and the cat. Moreover, much of the work since then has been based on their interpretations.

The preliminary study herein reported includes the ovaries of fourteen kittens of 3 weeks of age and is a description of the abnormalities occurring at this stage of development.

MATERIAL AND METHODS

Fourteen kittens, obtained from various sources, furnished the material for these observations. Ten of these kittens were 21 days old (i.e., 21 days after birth); the other four were between 15 and 28 days old.

Most of the animals were killed by a sharp blow on the head; three were anesthetized. The ovaries were removed as quickly as possible and fixed in Bouin's or strong Flemming's solution.

Longitudinal serial sections were cut $10\ \mu$ thick. These were stained with iron alum haematoxylin (Heidenhain's) and counterstained with eosin, Congo red or orange G. Congo red was preferred inasmuch as it served to bring out the idiozome.

Photomicrographs were made with a Zeiss apochromatic, 2 mm. oil immersion objective, N.A. 1.3; $12.5\times$ compensating ocular; and a Bausch and Lomb aplanatic condenser. Eastman DC ortho plates or commercial panchromatic film were used with Wratten filters B and G.

OBSERVATIONS

The cytological abnormalities observed fall into the following groups: a) multipolar mitoses; b) bipolar mitoses exhibiting certain chromosomal irregularities; c) tetrad-like chromosomes; d) multinucleate cells; and e) pycnosis and vacuolization. The cells in which these abnormalities are found are identified as oocytes by virtue of their location several layers below the oogonia and also by their unusually large size.

Multipolar mitoses. Tripolar mitoses. The tripolar division figures are by far the most abundant of all the abnormalities observed. Some of the cells in which they occur show signs of degeneration, for example the loss of granulation of the cytoplasm (fig. 16) and the fuzzy appearance of the spindle fibers accompanied by intense staining on the part of the chromatin material. In others, however, the general appearance is normal except for the tripolar figure (fig. 10).

Most of the polycentric figures observed are metaphases. This might be due to a prolongation of the metaphase or to the fact that this stage is more easily identified in the sections.

However, early (fig. 14) and late (figs. 13, 15) tripolar telophases were also found. In one of the late telophases (fig. 13), the cell walls of the three daughter cells are formed and nuclear reconstruction has begun. But the real evidence that

one tripolar cell may divide into three cells is the presence of the spindle fiber remains and of the mid-body or Zwischenkörper in the center of the three-cell group.

Five- and seven-spindle figures. Several five-spindle figures were found. In every case, the spindles are connected so as to form a quadripolar figure—the fifth spindle forming a diagonal—as shown in the diagram of figure 1. The chromosomes are arranged along the five equators, as in figure 17. The cells are located well below the surface layer of the ovary and are two or three times larger than neighboring oocytes. The five-spindle cells observed show no signs of degeneration, other than the spindle figure.

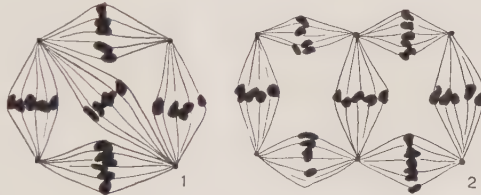


Fig. 1 Compare figure 17.

Fig. 2 Compare figure 7.

One seven-spindle metaphase was found (fig. 7). In this cell all of the seven spindles are connected through six centers (hexapolar) in the manner shown in figure 2. There are no apparent degenerative changes in the cytoplasm.

Still other, obviously multipolar cells were observed, but because of orientation in the sections or partial degeneration of the cell the spindle number could not be determined.

In every multipolar mitosis the chromosome distribution on the several spindles seems to be at random. An accurate count of the chromosomes is usually not possible but an estimate of the relative amounts of chromatin material on each spindle can be made. As the accompanying plates show, some of the spindles receive considerably more chromatin than others (figs. 7, 10, 11, 16, 17).

Frequently some of the chromosomes are not attached to the spindle in the usual fashion but one or more units are seen

in the cytoplasm, apart from the spindles (fig. 12). In at least two distinct cases, these isolated chromosomes are enclosed within a vesicular membrane (figs. 5, 16). On the other hand, the scattered chromosomes are occasionally attached by half-spindle fibers to the division figure.

Irregular bipolar mitoses. Many of the bipolar mitoses show irregularities in chromosome distribution. In some, the chromosomes are scattered quite at random along the spindle between the equator and the poles (fig. 12). In others, the chromosomes are scattered throughout the whole cell, some on the spindle, others apparently free in the cytoplasm and having no connection with the spindle. In still others, whole chains of chromosomes are set apart from the spindle. A very few cells were found in which there is a discernible fiber connection between the pole of the spindle and the aberrant chromosomes.

Tetrad-like chromosomes. The observation of tetrad-like chromosomes in certain mitotic figures indicates the occurrence of abnormalities in post-synaptic meiosis. That the cytological abnormalities recorded heretofore were related only to pre-synaptic meiosis is concluded from the fact that the chromosomes correspond in structure and number to those of somatic mitosis. Actual counts from polar views of metaphase plates gave between thirty-two and thirty-eight chromosomes per plate, whereas the expected diploid number according to von Winiwarter is thirty-six (haploid, eighteen).

The tetrad-like chromosomes, however, are morphologically like tetrad chromosomes of post-synapsis in that the number of units is smaller per figure, certain of these are definitely double (fig. 20), and typical cross, ring and V-shapes are present (fig. 8). Nevertheless, these chromosomes do not occur as they would at diakinesis, but are found on spindles. These spindles are not the kind one would expect if the divisions were premature maturation divisions. The spindles of the maturation divisions of mammals are usually barrel-shaped and devoid of centrioles, whereas these spindles were fusiform and had centrioles.

Multinucleate cells. Aside from the abnormalities relating directly to mitosis, numerous cells were observed which possess more than one nucleus within a single cell body. The number of these nuclei varies from two (figs. 4, 18) to nine, binucleate and trinucleate (fig. 6) being the most common. Whenever the staining and orientation are suitable, all of the nuclei within the cell seem to be quite definitely oriented to a single idiozome (fig. 18).

That all of the nuclei in a given multinucleate cell are always in the same stage of meiosis (figs. 4, 6) is significant. For example, certain cells contain several nuclei in the leptotene or pachytene stage with all of the threads showing the same amounts of chromaticity; other cells contain two or more nuclei in the late contraction stage.

This observation seems worthy of emphasis inasmuch as it offers evidence in favor of the view that multinucleate cells arise from the polycentric division of one cell, rather than from the confluence of several adjacent egg cells.

Pycnosis and vacuolization. Pycnosis or the condensation of the chromatin material in the nucleus is found throughout the cortical cords, sometimes in isolated cells, but more frequently in whole cysts of cells (fig. 19).

Less widespread than pycnosis is vacuolization of the cytoplasm, but it likewise often affects large groups of neighboring cells. One good example of this vacuolization is the binucleate cell (fig. 9) in which the entire cytoplasm is filled with vacuoles.

Both pycnosis and vacuolization are forms of cellular degeneration.

Series of degenerative changes. Since the nature of this material does not permit one to follow an individual cell through successive changes, any attempt to work out the origin and fate of the abnormal cells must necessarily be based upon serial arrangements of static pictures. Such an attempt has been made in the following series.

Irregularities of chromosome distribution along a bipolar spindle are the simplest form of mitotic abnormality observed.

The chromosomes do not distribute themselves properly and some are left off the spindle or lag along the spindle (figs. 12, 5). On division of the cell the daughter cells receive unequal shares of chromatin and degenerative processes leading to death ensue. In other words, those cells having incomplete chromatin complements probably cannot survive. If a cell having a vesicle containing extra chromosomes should divide, the daughter cell receiving the vesicle might be binucleate, the vesicle forming the extra nucleus.



Fig. 3 Diagram of cell shown in figure 16.

Multipolar mitoses probably arise whenever the dividing oocyte has more than two centrosomes. With three centers, tripolar spindles are formed and the chromosome distribution is at random and unequal (figs. 3, 16). Nuclear division of a tripolar cell may be accompanied by cell division so that three separate daughter cells are formed. Since these daughter cells could not receive normal chromosome sets in such a division, their nuclei eventually break down. Groups of pycnotic nuclei are evidence in favor of this conclusion.

On the other hand, if the nucleus divides without the cytoplasm, trinucleate cells are formed. Degeneration in this case

might appear as pycnosis of the nuclei or as vacuolization of the whole cell. While no trinucleate cell illustrating this was found, a binucleate cell was observed in which degeneration by vacuolization was taking place (fig. 9).

All cells with multipolar figures, regardless of the number of centers, probably proceed to degeneration in a manner similar to that outlined for those with tripolar figures. If this sequence is the correct one, the multinucleate cells would originate from a division of the nucleus unaccompanied by cytoplasmic division, rather than by confluence of adjacent cells.

The above series may not be the only valid ones for explaining the degenerative changes, but at least they include all of the observed abnormalities with the exception of the tetrad-like chromosomes.

Abnormal mitoses and multinucleate cells were described by von Winiwarter and Sainmont ('00, '09) in the oogonia of the rabbit and the cat during periods of regression of the medullary and cortical cords (35 to 45 days postpartum and following in the cat). Their cats were some 14 days older than those concerned in this study. The abnormal mitoses which they described were few in number and located in the peripheral extremities of the egg tubes; their fate was not evident but it was believed they did not contribute to the multinucleate cells and their derivatives. The multinucleate masses were considered to be degenerative phenomena. An arrest of the trophic role of the interstitial tissue was suggested as an explanation for the widespread degeneration of the presumptive gametes.

The abnormalities herein reported in the 21-day ovaries were more numerous than von Winiwarter and Sainmont have indicated in the reports of their material. Moreover, the abnormal cells were oocytes rather than oogonia. The fact that the divisions in these cells are abnormal confirms the view that, in general, oocyte differentiation is incompatible with normal mitosis. Abnormally induced divisions of the oocytes may, then, be the immediate cause for the degeneration of the primitive cortical cords in mammalian oogenesis.

SUMMARY

1. Certain abnormal nuclear phenomena of the oocytes in the ovaries of fourteen 3-week-old kittens are described. These consist of multipolar spindles, multinucleate cells, lagging and extruded chromosomes, tetrad-like chromosomes on somatic spindles, pycnosis and vacuolization.

2. An attempt has been made to arrange the various abnormalities in series suggestive of their origin and subsequent development.

3. That the abnormalities are in primary oocytes rather than oogonia was decided on the basis of the location and structure of the cells.

4. These abnormal nuclear phenomena are indicative of degenerative processes and undoubtedly contribute to the degeneration of the primary oocytes through the production of cells which are non-viable because of inadequate or otherwise abnormal chromatin complements.

I wish to take this opportunity to express my appreciation to Dr. James Walter Wilson under whose direction and inspiration this work was begun and pursued.

LITERATURE CITED

- WILSON, E. B. 1934 *The Cell in Development and Heredity*. 3rd ed., corrected. Macmillan Co., New York.
- VON WINIWARDER, H. 1900 *Recherches sur l'ovogenèse et l'organogenèse de l'ovaire des mammifères (Lapin et Homme)*. Arch. de Biol., T. 17, pp. 33-199.
- VON WINIWARDER, H., AND G. SAINMONT 1909 *Nouvelles recherches sur l'ovogenèse et l'organogenèse de l'ovaire des mammifères (Chat)*. Arch. de Biol., vol. 24, pp. 1-142, 165-276, 373-432.

PLATES

PLATE 1

EXPLANATION OF FIGURES

- 4 Binucleate and trinucleate oocytes in the same field. $\times 1500$.
- 5 Group of oocytes undergoing abnormal mitosis. Oocyte at upper right contains a vesicle with extra chromosomes. Mitosis at left is multipolar and very irregular. $\times 1820$.
- 6 Greatly enlarged trinucleate oocyte. Third nucleus in center (just out of focus) is smaller but in the same stage of meiosis as the other two. $\times 1500$.
- 7 Oocyte with seven spindles connected through six poles as in figure 2. $\times 1820$.
- 8 Greatly enlarged oocyte with tetrad-like chromosomes on a somatic spindle. $\times 1860$.
- 9 Binucleate oocyte in leptotene stage. The entire cell is vacuolized. $\times 1500$.

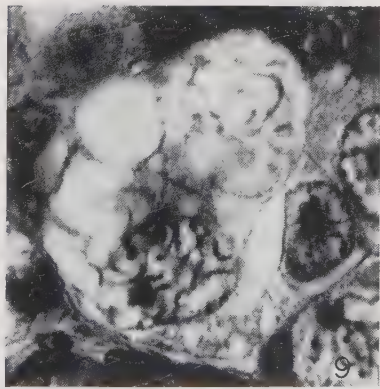
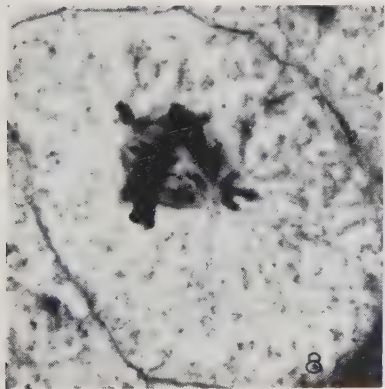
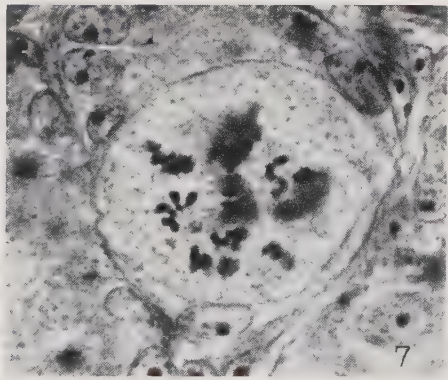
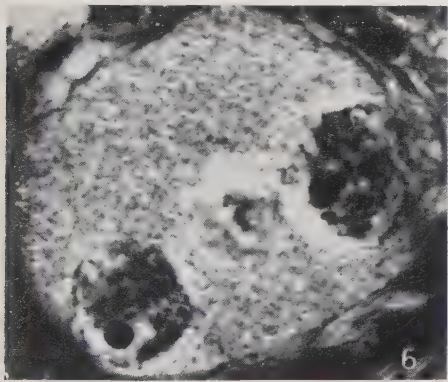
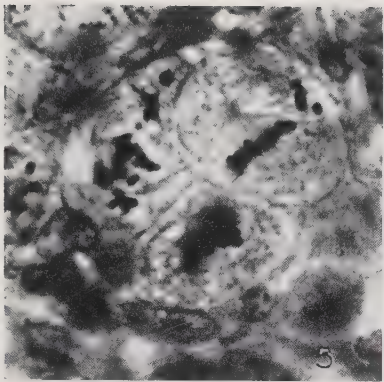
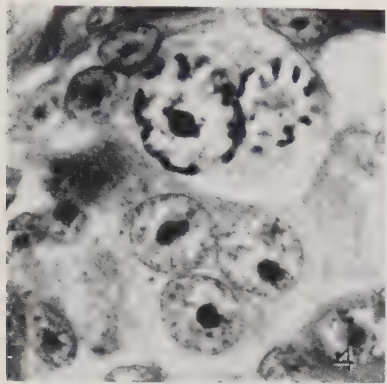


PLATE 2

EXPLANATION OF FIGURES

10 Oocyte with tripolar metaphase. Third pole at upper right is out of focus. $\times 2250$.

11 Oocyte with tripolar metaphase. Three or four chromosomes (at the right) are left off the spindle but are attached to it by half-spindle fibers. $\times 2250$.

12 Group of oocytes having bipolar spindles and scattered chromosomes. $\times 2250$.

13 Oocyte with late tripolar telophase. Three daughter cells are formed and nuclear reconstruction has begun. Spindle fiber remains and Zwischenkörper are evidence of the origin of three daughter cells from a tripolar mitosis. $\times 2250$.

14 Oocyte with tripolar telophase. $\times 2250$.

15 Oocyte with tripolar telophase in which cytoplasmic constriction has begun. $\times 2250$.

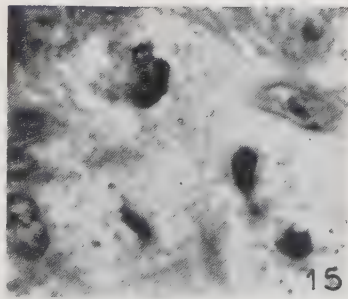
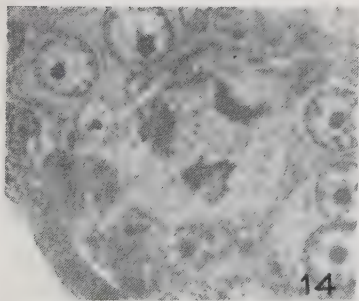
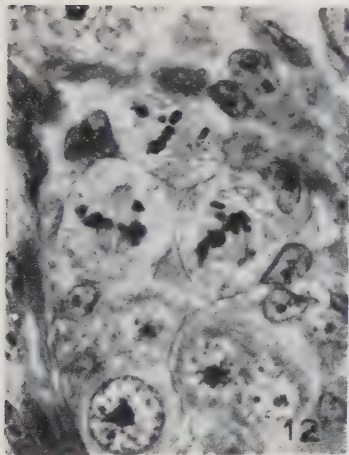
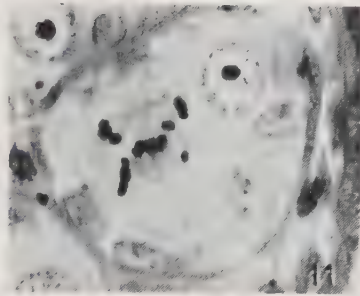
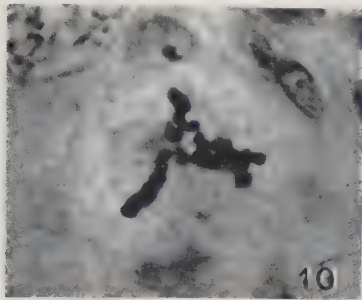


PLATE 3

EXPLANATION OF FIGURES

16 Oocyte with tripolar metaphase and a vesicle containing extra chromosomes (lower right). See figure 3. $\times 2250$.

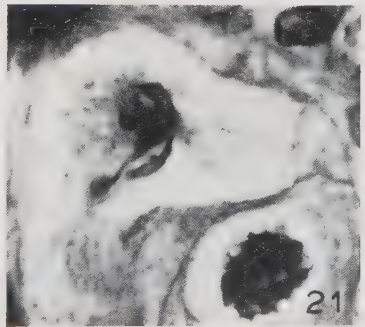
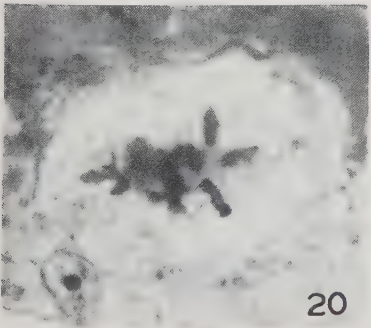
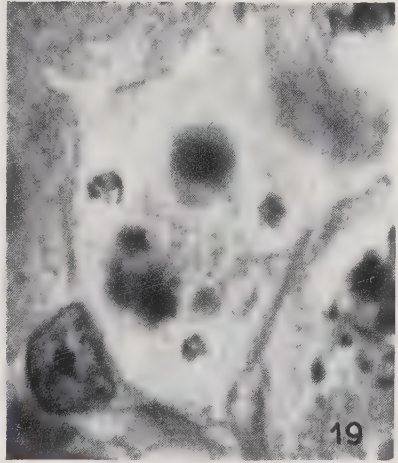
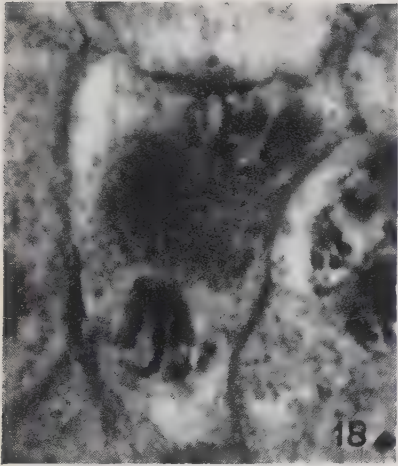
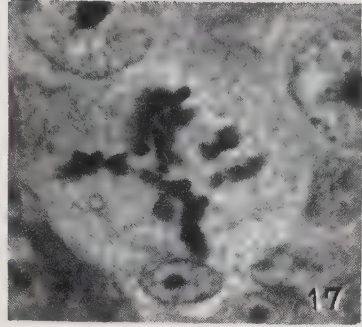
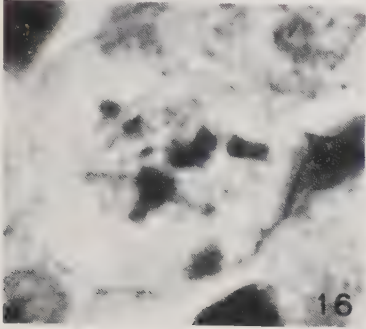
17 Oocyte with a quadripolar mitotic figure having five spindles. See the diagram in figure 1. $\times 2250$.

18 Binucleate oocyte in bouquet stage of meiosis showing orientation of the threads to a common, central idiozome (densely granular portion). $\times 2740$.

19 Multinucleate oocyte in which the nuclei are pycnotic. $\times 2250$.

20 Oocyte having a somatic spindle with tetrad-like chromosomes in which the double nature is very obvious. $\times 2250$.

21 Oocyte with tetrad-like chromosomes. Post-synaptic degeneration. $\times 1800$.



FUNCTIONAL HOMEOGRAFTS OF THE RAT ADRENAL GLAND GROWN IN VITRO

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THREE PLATES (EIGHT FIGURES)

From a survey of the literature, as well as from observations of our own, it seems to be established that an adrenal gland when excised from its own bed and transplanted to another part of the same body will grow and function. The medullary portion of the gland, as a rule, will not survive; but the cortical portion will regenerate and function indefinitely. The reader is referred to the excellent reviews by Jaffe ('27); Loeb ('30) and Britton ('30).

When the adrenal gland is transplanted to another animal (homeo-transplantation) the graft is less likely to be successful. When it is successful, a biologic or genetic identity in the cellular organization of the host and the graft may well be suspected. If close genetic relationships do not exist, then a cytologic reaction arises in the host against the graft, and the graft is likely to be destroyed within 30 to 40 days after transplanting (Loeb, '35, a, b; Loeb and King, '35). Although a homeograft may appear to be successful during the first few weeks after transplanting, disharmony between the host and the graft may arise, which results in an extensive lymphocytic infiltration ultimately destroying the transplant.

Because of this cellular antagonism on the part of the host toward a homeograft, some attempts have been made to change the organismal differentials of a tissue before transplanting it in order to adapt them to those of the host. Gassul ('22) and Erdmann ('27) showed that if skin of

certain *Amphibia* was grown *in vitro* and then transplanted to another species, successful grafts were obtained. Stone, Owings and Gey ('33, '34 a, b, c) applied this technic to the thyroid and parathyroid glands of mammals, including man. Subsequently, results of studies on the growth *in vitro* and transplantation of the pancreas (Murray and Bradley, '35; Selle, '35), pituitary body (Haymaker and Anderson, '36) and adrenal gland (Lux et al., '37) have been reported.

In our earlier paper (Lux et al., '37) we reported our observations on the growth of the adrenal glands of newborn animals *in vitro* and the results of transplanting these cultures to the groins of adult, normal, nonadrenalectomized rabbits. Animals which were grafted with the growing fragments had furnished the plasma and serum in which the fragments had grown. These grafts were completely destroyed in 12 weeks after transplantation.

The purpose of our present study was threefold. We wished to know first, whether adrenal fragments when grown outside of the body for a period of time would function and sustain life when transplanted to an adrenalectomized animal. Second, would adrenal fragments when grown in the sera of animals to which they were to be grafted survive and function any better after transplanting than those grown in the sera of some other, unrelated animal? And third, does the success of an adrenal graft depend on a physiologic need for the principle elaborated by the adrenal gland?

METHODS

The adrenal glands were removed aseptically under ether anesthesia¹ from white rats 1 to 2 days after birth. These glands were placed in Tyrode's solution and cut into fragments about 0.5 mm. in diameter. Five cubic centimeters of blood were taken by cardiac puncture from healthy, unrelated, male rats, and plasma was prepared. Embryo extract was made from 7-day-old chick embryos. The growth media consisted of 3 parts of embryo extract and 1 part of plasma.

¹ By Dr. Dwight Ingle.

Both Carrel flasks and Maximow slides were used. Eight to twelve fragments were placed in each flask and all cultures were washed and fed with fresh media every 2 or 3 days. All cultures were incubated at 37.5°C.

Cultures grown on Maximow slides were removed at intervals following planting, fixed in Carnoy's fluid and stained with dilute hematoxylin. Cultures grown in flasks were incubated for 10 days and then carefully removed and transplanted to the groin of an adult rat from which the adrenal glands had been removed. Twenty rats were grafted with fragments of growing adrenal tissue. Sixteen of these were grafted with fragments which had grown in their own sera and four were grafted with fragments which had grown in foreign sera. Cortin was administered to each rat for the first few days following the operation.

In order to secure histologic data on the appearance of the grafts three animals were killed at varying intervals after the transplantation. The grafts were removed, fixed in formalin and stained with hematoxylin and eosin, Mallory's connective tissue stain, Pap's stain for reticulum, for fat and for the chromaffin reaction. The grafts were aseptically removed, under anesthesia, from seven animals which lived 5 months after transplanting and the survival time of these animals was noted. All such grafts were fixed and prepared for histologic study.

OBSERVATIONS

1. *The growth of newborn rat adrenal in vitro.* Growth of the fragments was grossly visible in 18 to 24 hours after planting. Fibroblasts appeared first, as a rule, but epithelial cells were seen in many cultures at the end of 24 hours. Macrophages grew out about the third day and were abundant after the fifth or sixth day. The rate of growth of these three types of cells varied. Often in cultures in which fibroblasts had appeared first, the zone of epithelial growth soon exceeded the zone of fibroblasts. In other cultures fibroblasts often overgrew the other cells, so that it became difficult to

identify the sheets of epithelium. In many cultures one portion of the zone of growth was composed entirely of fibroblasts, while epithelium grew from another portion of the same fragment (fig. 1).

Epithelium of the growing adrenal fragment usually grew out as a sheet, comprised of small, irregular cells with definite intercellular spaces (figs. 2 and 3). Fibroblasts ordinarily appeared as elongated, spindle-shaped cells, but on the surface of the clot they grew as a syncytium and resembled somewhat the epithelial pattern of growth.

2. The results following transplantation of the growing adrenal fragments. Of the twenty adrenalectomized rats which were grafted with fragments of newborn rat adrenals grown in vitro, grafts were successful in ten. Seven of these ten animals had functional adrenal grafts 5 months after transplantation (fig. 4). Of these seven animals, six had received fragments which had been cultured in media containing their own sera; while one had received fragments which had been cultured in media containing foreign sera. Ten animals, seven of which had received fragments cultured in their own sera and three of which had received fragments cultured in foreign sera, died during the first 6 weeks after transplanting, of adrenal insufficiency.

All seven animals which survived 5 months after transplanting the fragments, went into adrenal insufficiency and died within 2 weeks after the grafts were removed. This indicates the functional capacity attained by these transplanted cultures.

Although all adrenal glands were cultured substantially in the same way, and all grew more or less alike, there were marked differences in the character of the host reaction toward these adrenal cultures after they were transplanted to the groin. Grafts which grew and became functional adrenal glands (fig. 5) did not elicit a lymphocytic or giant cell reaction such as were found in those grafts (fig. 6) recovered from animals which died of adrenal insufficiency in the first 6 weeks after grafting.

Although as many as thirty or forty fragments of growing adrenal tissue were transplanted to the groin, only a few well-defined cortical nodules developed. These nodules (fig. 4), composed of compact cortical tissue, were formed by the fusion of several of the originally discrete adrenal fragments; for strands of capsular tissue within the nodule suggested its multiple origin. These nodules were always well vascularized (fig. 5) and the amount of lymphocytic and leukocytic infiltration was negligible in those grafts which were successful. The adrenal cells of these regenerated cortical nodules were larger than in an adult gland, the nuclei were large and the increase in size and number of the nucleoli characterized a rapidly growing tissue.

Although the size and shape of these regenerated cortical masses resembled those of a normal adult adrenal gland, the histologic pattern was different. A well-organized medulla never was seen. The chromaffin stains revealed small areas of medullary tissue scattered throughout the cortical masses, but the amount in comparison with that of cortical tissue was exceedingly small. An extensive reticular framework, which supported the cords or columns of cells, developed with the cortical tissue, so that a gland which had regenerated from the fragments grown in vitro 5 months after transplantation had a supporting framework quite like that of a normal adult gland (fig. 7).

Each well-defined cortical nodule examined from 1 to 5 months after transplantation was enclosed by a capsule. Beneath this capsule there was an actively proliferating glomerular zone and beneath that a region which could well be compared with the zona fasciculata of the normal gland. As in the normal gland, the cells of the glomerular zone in these regenerated nodules were small, their nuclei were hyperchromatic and their cytoplasmic bodies were correspondingly small. They always contained varying amounts of lipid.

The growth of these nodules appeared to be from the outer glomerular zone toward the center, just as has been shown in the normal development of the adrenal gland. Beneath the

glomerular zone the cells increased in size, large lipoid droplets were formed and typical spongiocytes appeared (fig. 8).

Zwemer ('36) was of the opinion that the capsule of the adrenal gland gave rise to the cortical cells by a progressive differentiation. On section of our regenerating nodules the same relation was revealed between the outer capsule and the zona glomerulosa that Zwemer has described for the normal, adult gland. One may identify all gradations in shapes of cells, from the elongated, definitely capsular cell, through the ovoid forms, to the spherical cells of the glomerular zone. There were often centers of proliferation in the capsule and as a result clusters of glomerular cells developed (fig. 8). It is of biologic interest that diffusely growing fragments of cortical tissue transplanted to the groin should assume so compact a nodular form, with capsule and other anatomic characteristics of a normal gland.

COMMENT

The adrenal glands of newborn white rats grow exceedingly well *in vitro* and when such cultures are transplanted to the groins of adrenalectomized adult rats they may grow, elaborate the principle of the adrenal gland and maintain the life of the animal. Function of these cultures which grew into definite cortical nodules in the groin was clearly established, for when they were removed 5 months after transplanting, the animals all developed adrenal insufficiency and died.

The success or failure of these adrenal grafts did not appear to depend on the character of the media in which the fragments had grown prior to transplantation. In other words, growing fragments in the fluids of an animal, prior to transplanting them to that animal, did not insure successful grafts. Ten of the animals died of adrenal insufficiency, within 3 to 4 weeks after they were grafted, and yet seven of them had received fragments which had grown for 2 weeks in their own body fluids. Three of the ten animals which died of insufficiency had been grafted with fragments which had been cultured in another animal's serum.

Cytologic reactions by the host against the grafts were evident in all animals which died of insufficiency, whether the fragments had grown in media containing the animal's own sera or not. Thus, by the culture method we apparently had not changed the biologic characteristics of the graft and had not made them compatible with those of the host. Against some grafts the lymphocytic action of the host was much greater than against others. This severe lymphocytic infiltration indicated, we believe, a wider genetic differentiation between donor and host than existed when the reaction was less severe.

The presence of large numbers of lymphocytes in the unsuccessful grafts, although grown in the fluids of the recipient animal, suggests, at least, that a successful graft was probably not the result of a process of acclimatization, attempted by tissue culture methods. But successful grafts, we believe, were more likely attributable to some definite biologic or genetic compatibility between the graft and the host, a condition which existed even before culture of the glands in the fluids of the host was undertaken.

The presence or absence of the principle elaborated by the adrenal gland had an important bearing on the success or failure of an adrenal graft. Halsted ('09), it will be recalled, suggested the 'law of deficiency' wherein he stated that there must be a physiologic need for a glandular principle in order that a graft of the gland elaborating that principle be successful. When we had grafted the adrenal gland to the groin of nonadrenalectomized adult rabbits (Lux et al., '37) we had not secured a single successful graft. On the other hand, when a deficiency of the adrenal principle was induced in adult rats before grafting, as by bilateral adrenalectomy, a large percentage of successful grafts was obtained.

SUMMARY

This report concerns the results of homeografting newborn rat adrenal fragments, grown in vitro, to unrelated, adrenalectomized, adult rats. Twenty adult rats were grafted. Sixteen of these received fragments cultured in their own body

fluids and four received fragments cultured in body fluids of another animal. The conclusions indicated are as follows:

1. The adrenal gland of the newborn rat may be grown in vitro and transplanted to adrenalectomized rats, where it may grow and function.

2. Ten grafts of these adrenal fragments were successful and ten were failures.

3. Of the ten successful grafts, nine previously had grown in the recipient's own fluid and one had grown in the fluids of another animal.

4. Of the ten grafts which failed to survive, seven had grown in the recipient's own fluid and three had grown in the fluids of another animal.

5. Previous growth of adrenal fragments in fluids of the animal to which they were to be grafted does not insure survival of grafts.

6. Lymphocytic infiltration and giant cell reaction by the host against the graft may completely destroy the graft even though the fragments were grown in the host's body fluids.

7. Successful grafts were probably the result of a biologic or genetic identity between donor and host rather than the result of any change in the fragments induced during their growth in vitro.

8. Adrenalectomy, resulting in a deficiency of the adrenal principle available to the organism, is essential for the success of an adrenal graft.

9. It may be that further study will show that changes in tissues may be produced by growth in vitro but as far as this study is concerned the evidence is not entirely convincing that the biologic differentials existing between host and donor may be modified during the period of growth in a tissue culture media.

LITERATURE CITED

- BRITTON, S. W. 1930 Adrenal insufficiency and related considerations. *Physiol. Rev.*, vol. 10, pp. 617-682.
- ERDMANN, RHODA 1927 Verwandtschaftsbeziehungen der Anurenfamilien, geprüft durch implantationsversuche gezüchteter Haut. *Arch. f. Entwickl. d. Organ*, Bd. 112, S. 739-806.
- GASSUL, R. 1922 Homoplastische transplantation von explantaten aus erwachsener Froschhaut. *Deutsch. med. Wchnschr.*, Bd. 48, S. 1163-1164.
- HALSTED, W. S. 1909 Auto- and isografting, in dogs, of the parathyroid glandules. *J. Exp. Med.*, vol. 11, pp. 175-199.
- HAYMAKER, WEBB AND EVELYN ANDERSON 1936 Homiograftering of rat pituitary grown in vitro. *J. Path. and Bacteriol.*, vol. 42, pp. 399-410.
- JAFFE, H. L. 1927 On the transplantation of guinea pigs suprarenal and functioning of the grafts. *J. Exp. Med.*, vol. 45, pp. 587-594.
- LOEB, LEO 1930 Transplantation and individuality. *Physiol. Rev.*, vol. 10, pp. 547-616.
- 1935 a Comparison of the reactions against heterotransplanted tissues in different kinds of hosts. *Biol. Bull.*, vol. 68, pp. 440-450.
- 1935 b Transplantation of tissues from mouse to rat and vice versa. *Am. Nat.*, vol. 69, pp. 239-244.
- LOEB, LEO, AND H. D. KING 1935 The analysis of the organismal differentials of gray Norway rats and of two mutant races by means of transplantation. *Am. Naturalist*, vol. 69, pp. 1-18.
- LUX, LYDIA, G. M. HIGGINS AND F. C. MANN 1937 Homeotransplantation of the guinea pig and rabbit adrenals grown in vitro. *Anat. Rec.*, vol. 67, pp. 353-365.
- MURRAY, MARGARET R., AND C. F. BRADLEY 1935 Two island-cell adenomas of the human pancreas cultivated in vitro. *Am. J. Cancer*, vol. 25, pp. 98-107.
- SELLE, W. A. 1935 Studies on pancreatic grafts made with new technique. *Am. J. Physiol.*, vol. 113, pp. 118-119.
- STONE, H. B., J. C. OWINGS AND G. O. GEY 1933 Living grafts of endocrine glands. *California and West. Med.*, vol. 38, pp. 409-411, and vol. 39, pp. 10-12, 81-83.
- 1934 a Transplantation of living grafts of thyroid and parathyroid. *Lancet*, vol. 1, pp. 625-626.
- 1934 b Living grafts of endocrine glands. *Am. J. Surg.*, vol. 24, pp. 386-395.
- 1934 c Transplantation of living grafts of thyroid and parathyroid glands. *Ann. Surg.*, vol. 100, pp. 613-628.
- ZWEMER, R. L. 1936 A study of adrenal cortex morphology. *Am. J. Path.*, vol. 12, pp. 107-114.

PLATE 1

EXPLANATION OF FIGURES

- 1 Eight-day living culture of the adrenal gland of a newborn rat, grown in a D-5 flask. $\times 40$.
- 2 Six-day living culture of the adrenal gland of a newborn rat, grown in a D-5 flask. $\times 100$.
- 3 Eight-day fixed and stained culture of adrenal gland of a newborn rat, grown on Maximow slide. $\times 125$.

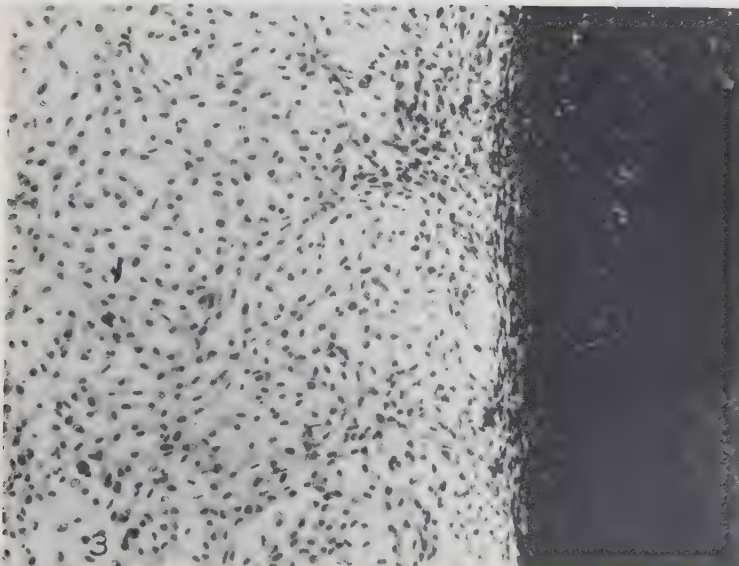
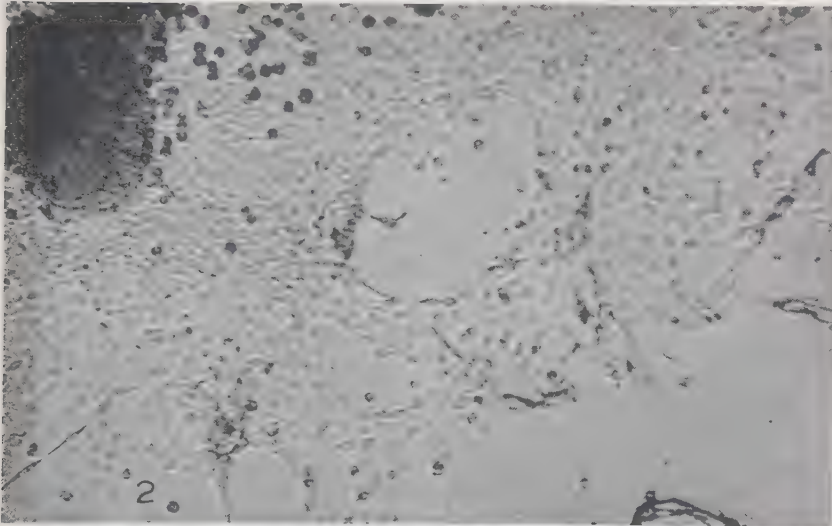
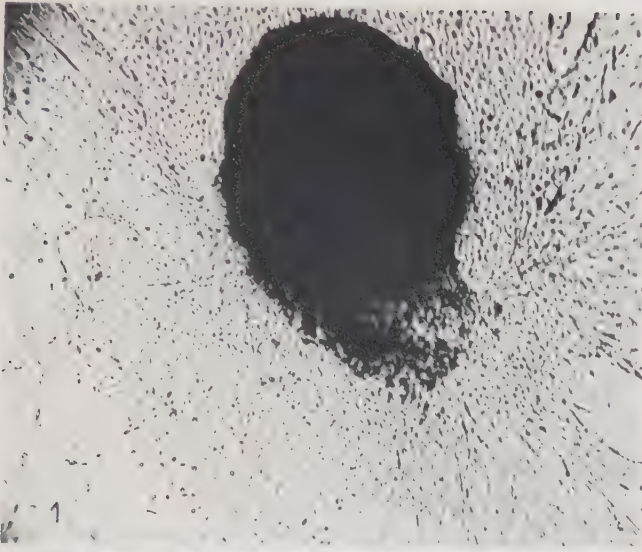


PLATE 2

EXPLANATION OF FIGURES

4 Cluster of adrenal glands in groin of rat 5 months after transplantation of fragments grown in vitro. Recipient had been adrenalectomized.

5 Adrenal gland recovered from groin of a healthy, adrenalectomized adult rat, 1 month after transplanting fragments grown in vitro. The vascularity, capsule, zona glomerulosa and zona fasciculata can be seen. $\times 47$.

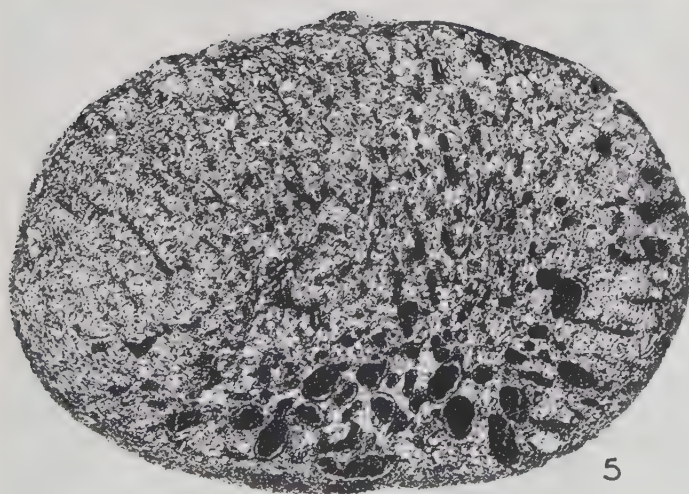


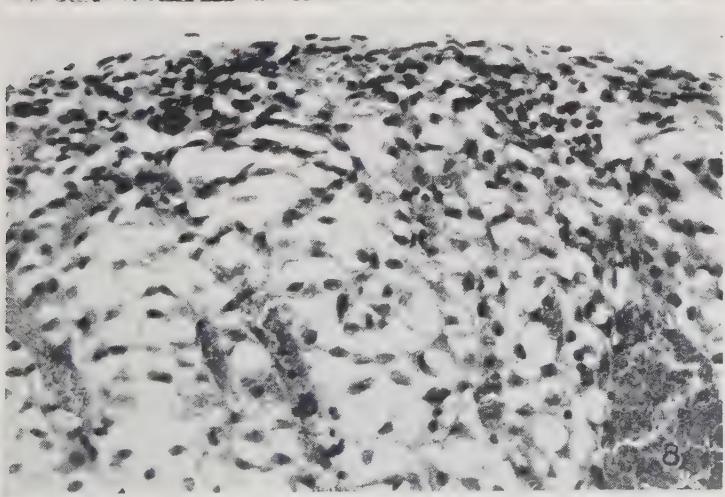
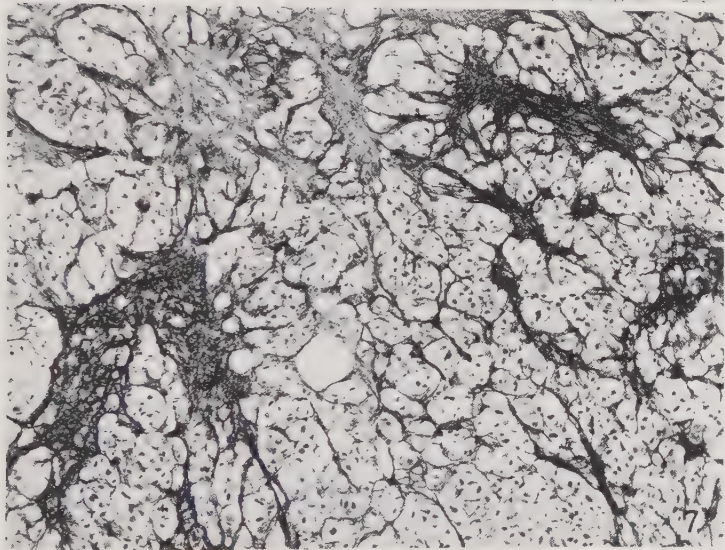
PLATE 3

EXPLANATION OF FIGURES

6 Transplanted fragments removed from groin of an animal dying from adrenal insufficiency 16 days after grafting. Infiltration, giant cells and general destruction of graft can be observed.

7 Cortical nodule recovered from groin of adrenalectomized adult rat, 5 months after transplantation showing reticular fibers. Silver impregnation stain. $\times 95$.

8 Section through margin of cortical nodule recovered from groin 5 months after transplantation. Thickness of capsule and regions of proliferation beneath capsule to form cells of zona glomerulosa may be noted. $\times 285$.



THE ORIGIN OF THE SYMPATHETIC TRUNKS IN THE CHICK EMBRYO ¹

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FOUR TEXT FIGURES AND TWO PLATES (TWELVE FIGURES)

INTRODUCTION

In recent years two opposing theories have arisen to explain the origin of the sympathetic nervous system in chick embryos—the warm-blooded forms most available for experimental study. The first view—illustrated by the works of Müller and Ingvar ('23) and of Van Campenhout ('31)—maintains that the sympathetic trunks originate exclusively from the spinal ganglia. The second, supported largely by the studies of Kuntz ('10, '22, '26), holds that the sympathetic system arises mainly from neuroblasts located in the ventral portion of the neural tube. Both theories have been based upon the study of normal stages in development and upon the examination of embryos submitted to experimental procedures. Of these two methods, the former is less satisfactory since at the time of active migration of sympathetic neuroblasts (third day of incubation) the ventral extremity of the spinal ganglion has grown forward as far as the ventral root, and it is impossible to determine from which source the cells are derived. Accordingly, the question must be settled by experiment.

In the present study, two types of operations have been undertaken: first, removal of the neural crests and adjacent portions of the neural tube; second, removal of the crests and whole neural tube in a given region—it being mechanically

¹ Thesis submitted in partial fulfillment of the requirements for the degree of doctor of philosophy under the direction of Prof. E. A. Boyden. The author is now a member of the Department of Anatomy, Loyola University Medical School, Chicago, Ill.

impossible to extirpate the tube and leave the crests. Thus, by removing one of the sources from which the sympathetic system might arise, in one series of embryos, and by removing both of the possible sources in another group of embryos, it was hoped that critical information might be obtained.

MATERIAL AND TECHNIQUE

The two types of operations mentioned above were carried out on chicks that had been incubated 40 to 48 hours. At this period the neural tube in the region of the vitelline arteries has just closed thereby affording an opportunity for the removing of the neural crest during its incipient stage of development. In the first series of embryos the crests at or below the level of the vitelline arteries were removed for a distance of from two to seven segments. This operation was performed by incising the ectoderm longitudinally along either side of the axis, and then cutting off a small amount of tissue from the dorsal part of the neural tube. Thus, the dorsum of the spinal cord and the ectoderm overlying it were completely removed. In another series of embryos, the ectoderm was cut as before, following which a whole segment of the neural tube was removed together with the overlying ectoderm. Then the embryos were reincubated for from 1 to 5 days, fixed in 95% alcohol with 10% chloral hydrate, stained in toto by the Cajal technique, and sectioned serially.

The details of the operative technique were as follows: after the required hours of incubation, the egg was removed from the incubator and the position of the embryo determined by a candling lantern in a semi-dark room. Then the surface of the egg over the embryo was quickly wiped with 70% alcohol, following which a hexagonal piece of shell about $1\frac{1}{2}$ cm. across was removed by means of a dental drill. This piece of shell was dipped in alcohol and placed on sterile gauze. A wall of paraffin was then built up around the opening in the egg shell forming a shallow depression which was filled with sterile Ringer's solution at the proper temperature. The shell membrane was cut near the edges of the opening and removed.

The egg was then placed in a plaster cast about 3 cm. deep which, in turn, rested upon a warming plate kept at incubation temperature. A binocular dissecting microscope was adjusted over the plate and a pencil of light shot in from the side. The saline floated the embryo up to where it could be illuminated. Then the desired portions were excised with sterile iridectomy scissors, following which the saline was removed, the piece of shell replaced, and sealed in position by a mixture of resin and beeswax. The process required from 10 to 30 minutes, the more rapid procedure being the more satisfactory.

DISCUSSION OF EXPERIMENTAL DATA

The experimental material available for study consisted of twenty-five embryos, twelve of which, embodying all essential points to be found in the series, were selected for graphic reconstruction. The general level of the area of operation is shown in figure 1 (D to Z). Longitudinal views of the cord and sympathetic trunks in these twelve embryos are presented in the various text figures.

The specimens fall into two groups: 1) thirteen embryos in which only the neural crests and contiguous portions of the neural tube were removed; and 2) twelve embryos in which the neural crests and whole segments of the tube have been excised.

Embryos of group 1

The first group, as indicated in figure 2, show that the sympathetic trunks will develop in an area from which the neural crests have been removed. Thus, in chick I the trunks are normal in appearance with characteristic distribution of ganglia, yet the spinal ganglia are absent on both sides for three segments. On the left side of chick D seven spinal ganglia are missing, yet the sympathetic trunk is present. It is obvious that the embryos of group 1 refute the statement of Müller and Ingvar, namely, that after removal of the neural crests no sympathetic ganglia form, regardless of the condition of the neural tube.

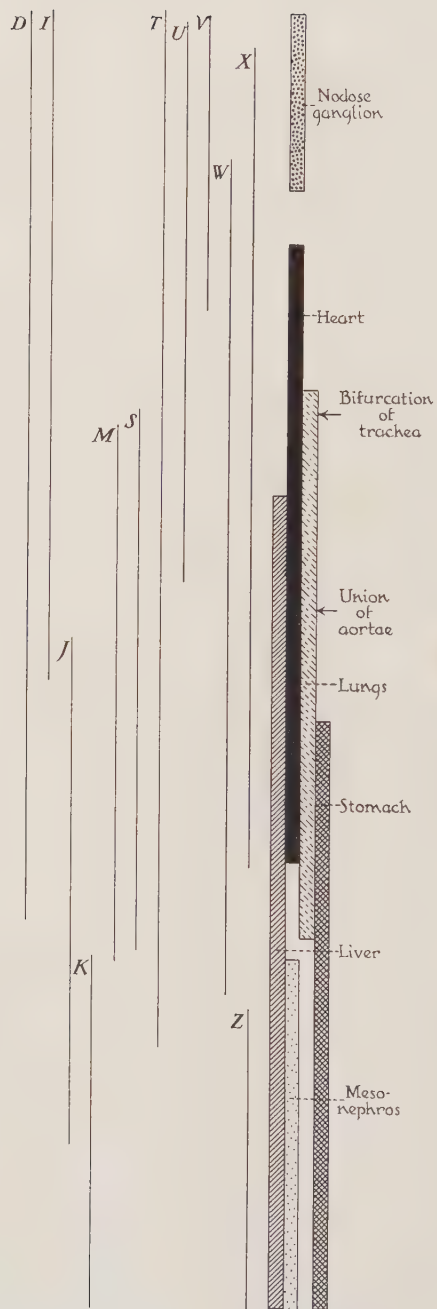


Fig.1 Chart showing approximate level of operations in relation to various organs (based on 7-day chick).

The second embryo of group 1, chick K (figs. 2 and 7) is quite similar to chick I, except that more of the roof of the medullary tube was removed so that there is less of the spinal cord present. Apparently, this increased loss of nervous tissue from the spinal cord has affected the development of the sympathetic elements, for the sympathetic trunks are not as symmetrical as in chick I nor are the ganglia distributed so regularly.

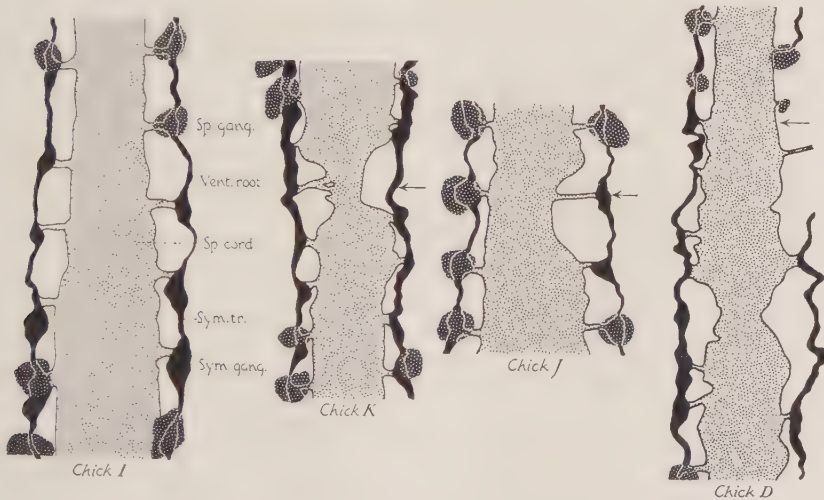


Fig. 2 Embryos of group 1 (7-day chicks). Graphic reconstructions of the nervous system in the region from which neural crests and the dorsal portion of the neural tube was removed at the 40- to 48-hour stage of development.

In the third embryo of group 1 (chick J) it is evident that the more cephalic sympathetic ganglion in the operated area is smaller than its mate of the opposite side. Sections at this level (fig. 10) show that the side of the neural tube near the smaller ganglion is practically missing, while the ventral root has grown out from the opposite side of the neural tube. Note that the left side of this embryo displays the normal relationship of spinal cord, spinal ganglia, and sympathetic trunks.

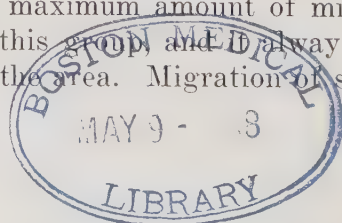
In the fourth embryo in group 1, chick D, the neural tube was considerably damaged in the upper right end of the area

of operation, and has undergone degenerative changes (fig. 6). On this side the sympathetic trunk is interrupted in the area where the tube was more or less severely damaged. In this same area there is a diminutive spinal ganglion indicating that the ventral portion of the neural crest had been left there at the time of the operation. Yet no sympathetic ganglion has grown out of it.

Thus the embryos of group 1 show unmistakably that sympathetic trunks will develop in an area from which the neural crests have been removed, and that the formation of the trunks in such an area is correlated with the condition of the neural tube. These conditions create a strong presumption that the sympathetic ganglia are derived from cells that migrate from the ventral portion of the neural tube. On the other hand, the possibility remains that the cells may have migrated from neural crest elements at the ends of the operated zone; or cells which are to form the sympathetic ganglia may have migrated from the neural crests before the operation, as a recent work of Detwiler indicates in *Amblystoma*. These possibilities will be discussed later.

Embryos of group 2

These embryos consist of specimens from which the whole of the neural tube has been removed for a distance of from one to seven segments. They show that if more than three segments of the neural tube are removed, the sympathetic trunks will fail to develop in that area (figs. 3, 11, 12). It is apparent, however, that sympathetic elements will migrate into the area from which the neural tube has been removed. This migration varies for from one to five segments (fig. 3) and proceeds into the area from the cephalic end. Although in chick T (fig. 3) the sympathetic trunks have proceeded into the area for five segments, the sympathetic ganglion cells themselves have migrated only three segments. In fact, three segments is the maximum amount of migration exhibited in any embryo of this group and it always proceeds from the cephalic end of the area. Migration of sympathetic cells for



three segments occurred in two of five embryos of this type. In two others, the migration was for one segment and in a fifth embryo, slightly over two segments. No appreciable amount of migration occurred from the caudal end. These facts become highly significant when it is recalled that in chick D of group 1 the sympathetic trunk is present in a region from which seven consecutive spinal ganglia are missing.

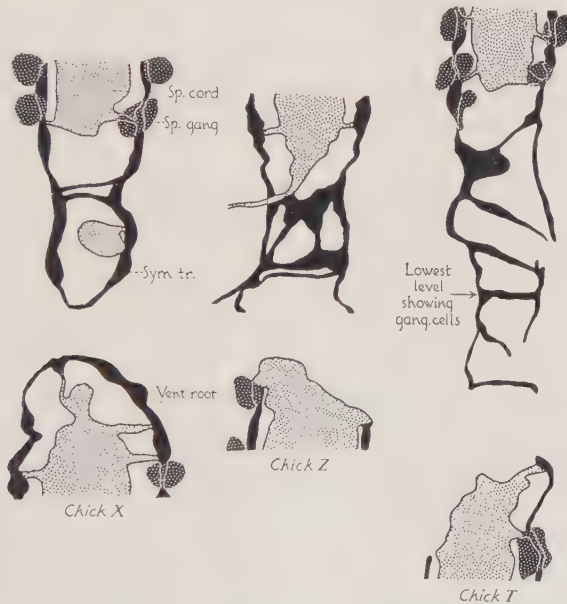


Fig. 3 Embryos of group 2 (chick W, fig. 12, also belongs in this group). Graphic reconstructions showing the effect of removing whole segments of the neural tube in addition to the neural crests.

This sympathetic trunk could not have arisen from cells moving into the area from the ends of the zone of operation unless the migration proceeded twice as far as that demonstrated by any of the embryos of group 2. The obvious conclusion is that the sympathetic ganglia in chick D were formed by neuroblasts which came from the ventral portion of the neural tube.

The ability of sympathetic elements to migrate into the cephalic end of the wound makes it possible for the sympathetic trunks to form in an area from which a small section

of the neural tube has been removed, as in chicks S, M, V, U (fig. 4). The variability of the migration is exemplified by the fact that in chick W (fig. 12) the sympathetic trunks did not bridge a gap in the neural tube of slightly more than two segments, while in chick T (fig. 3) the trunks extended into the zone of operation for five segments. In chick U (fig. 4) the trunks have bridged a gap of five segments, but in this case a piece of neural tube which sprouted ventral roots was left in the zone of operation.

Chick S of this group affords further evidence that cells which traverse the ventral roots from the neural tube form

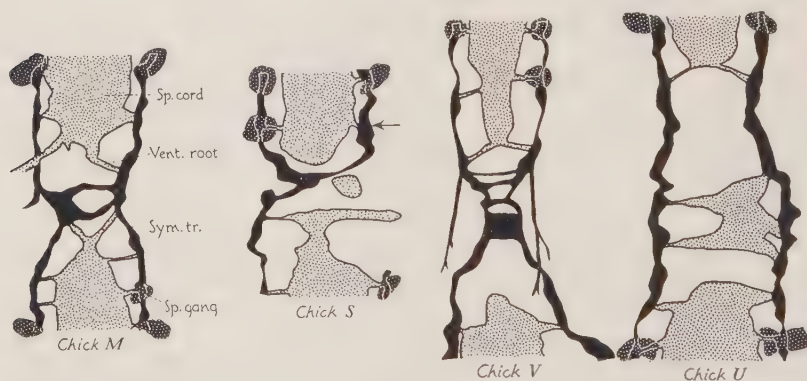


Fig. 4 Additional embryos of group 2, showing the result of less extensive removal of the spinal cord.

the sympathetic ganglia. Figure 13 is a photomicrograph of a section at the level indicated in figure 4. Examination of this section and neighboring ones reveals that the ventral root is a fibrillo-cellular extension of the ventral part of the spinal cord. A short distance from the spinal cord—as indicated by the high power view (fig. 9)—it has the typical appearance of a sympathetic ganglion (compare with fig. 8). Indeed it is continuous around the cartilage, with the more distally placed sympathetic ganglion lying ventrolateral to the cartilage containing the notochord (fig. 13). This connection must be interpreted either as the persistence of the earlier 3-day stage of development (fig. 15) or as a result of secondary fusion of the

spinal cord with a sympathetic trunk that had grown down into this region from above. Since the ventral roots in the 3-day chick are normally crowded with neuroblasts, as will be demonstrated later, the more natural interpretation is that this condition represents a persistence of an earlier stage in normal development.

STAGES IN NORMAL DEVELOPMENT OF SYMPATHETIC SYSTEM

The migration of neuroblasts into the ventral roots

If it is true that neuroblasts which migrate from the neural tube form the sympathetic system, as indicated above, then study of the normal stages in development should reveal these neuroblasts in the ventral roots. Figure 15 shows the migrating phase in a 3-day chick. In the upper left corner may be seen the cells of the spinal ganglion (s.g.), in the upper right corner the neuroblasts of the spinal cord (s.c.) and in between the two the darker staining strands of the ventral root (v). Within this root may be seen slightly oval nuclei that closely resemble those in the neural tube. Discounting the shrinkage space between the neural tube and surrounding structures there is an unbroken continuity between this chain of cells in the ventral root and the cells in the neural tube. Although this condition has been described by Froriep, Cajal, Ganfini, Rau and Johnson, Uchida, Kuntz and others, Müller and Ingvar state that neither during the normal developmental period of the spinal nerves in the chick, nor in the operated cases, does an out-wandering of medullary cells along the ventral roots take place.

Van Campenhout ('31) describes cells in the ventral roots of the 3-day chick which he interprets as arising from the spinal ganglia and migrating up the ventral root toward the neural tube to form sheath cells on the fibers of the ventral root. These cells are elongated cells identical to cells which migrate out from the dorsal extremities of the spinal ganglia along the dorsal roots. Concurrently, he describes a conspicuous migration of large rounded neuroblasts, with deeply stained nuclei, from the ventral extremity of the spinal ganglion. He considers these to be the cells which will give rise

to the sympathetic system, and states that they may be distinguished from the elongated pre-sheath cells by their large rounded form and deeply staining nuclei. Raven ('37), however, has shown that in the amphibian, contrary to the long accepted work of Harrison, the sheath cells are derived from cells of medullary origin which migrate along the ventral roots.

I cannot confirm the description given by Van Campenhout. As seen in figure 15, the cells within the ventral root (v.) are quite similar to the neuroblasts in the neural tube (s.c.). Although a bit oval in shape they are not so elongated as to be readily distinguished from neuroblasts. Furthermore, the ventral roots have the same appearance as in an embryo from which the neural crests have been removed. Such a specimen is shown in figure 16. Note that there is no spinal ganglion in the section and that the dorsal portion of the neural tube has been removed. Yet the ventral root contains as many cells as the ventral root of the normal embryo.² There is no escaping the conclusion, therefore, that these cells are of medullary origin and comprise the elements responsible for forming the sympathetic trunks in the embryos from which the neural crests have been removed. This being the case, it is logical to assume that normally the sympathetic system results from the differentiation of neuroblasts which have migrated from the neural tube along the pathway of the ventral roots.

The formation of primary and secondary trunks

His, Jr. (1897) described two pairs of sympathetic trunks which he designated as primary and secondary. According to his description, the primary trunks arise about the close of the third day of incubation as a pair of cell columns lying along the sides of the dorsal surface of the aorta. About the beginning of the sixth day, the primordia of the secondary trunks arise as cell aggregates situated just median to the

² The silver technique was used in this case because it differentiates the nervous and mesenchymal tissues better than does the cresyl violet stain used in figure 15.

ventral roots of the spinal nerves. The cell aggregates are at first independent of each other but become united later by longitudinal commissures. Between the fourth and eighth days the primary trunks disappear, except in the most anterior region, becoming resolved into the ganglia and nerves constituting the prevertebral and peripheral sympathetic plexuses. The cells giving rise to both primary and secondary sympathetic trunks were derived exclusively from the spinal ganglia.

The same general description regarding the formation of primary and secondary trunks is given by Kuntz ('10) except that he derives the cells from the neural tube instead of the spinal ganglia. According to Kuntz, there are two migrations of cells from the neural tube to form the primary and secondary trunks, respectively.

Van Campenhout ('31) and Müller and Ingvar ('23) describe only one migration of cells. These cells are said to migrate from the spinal ganglia and form all of the sympathetic elements. The primary trunks are formed about the close of the third day and the secondary during the fourth day of incubation.

My own observations confirm the description given by Van Campenhout, except that I would derive the cells from the neural tube. There appears to be only one migration of cells which form the sympathetic system. Both trunks are formed from these migratory cells. The migration takes place at about the end of the third day. At this stage the spinal ganglia have extended ventrally along the sides of the neural tube to meet the ventral roots. As stated above, the ventral roots are crowded with neuroblasts similar to those within the wall of the neural tube. It is this mingling of the cells of the spinal ganglion with elements of the ventral root which has led to the two opposing theories based on studies of normal embryos.

The sympathetic ganglia themselves appear first in the upper thoracic region and then develop progressively in both cranial and caudal directions. The primary sympathetic trunks form during the beginning of the fourth day on the

dorsolateral side of the aorta. Intersegmental connections develop between the ganglionic masses. The secondary ganglia are formed from the same group of cells which form the primary ones. There is no evidence that further migration from the central nervous system takes place, but the primary and secondary ganglia are intimately connected during the early development of the secondary ganglia. Indeed, the primary trunks seem to be incorporated into the secondary trunks and retain their interganglionic connections, while the secondary ganglia also establish fibrillar connections so that there are usually two or more nerve trunks between the ganglia of the permanent trunks.

DISCUSSION

The conclusion drawn from these observations and experiments is that the sympathetic trunks are formed from cells which migrate from the neural tube along the pathway of the ventral roots and communicating rami. As stated above, other possibilities must be considered. Is it possible, for instance, that the cells giving rise to the sympathetic ganglia may have migrated from neural crest elements at either end of the area of operation? Except for the evidence presented in this paper, no one has ever demonstrated satisfactorily that neuroblasts can migrate longitudinally. The embryos of group 2, however, reveal the fact that these cells can migrate caudally as much as three segments (two out of five cases). Yet this does not justify the assumption that neuroblasts could migrate far enough to fill a gap of seven segments.

Furthermore, there is evidence that the sympathetic ganglia of the region along the neural tube where the spinal ganglia are missing are not derived from cells that migrated longitudinally. Chick I (fig. 2) has the least injured neural tube of any of the embryos of the series. The sympathetic ganglia in this embryo are regularly distributed and exhibit the normal relationship to the ventral roots. In the sympathetic trunks which are the result of a migration of cells from the cephalic end of the area of operation (fig. 3) the ganglion cells

are not grouped in discrete ganglia but are scattered all along the trunks. The same condition prevails in those embryos of group 1 in which the neural tube was damaged considerably with consequent interference with the migration of cells along the ventral roots (chicks K and D, fig. 2). In chick V (fig. 4) the second ganglion from the top on the left is normal while ganglion cells extend irregularly along the trunk from the third ganglion. So it seems that if a sympathetic ganglion is formed from the cells which migrate out from one ventral root the ganglion will be normal, but if it results from cells which migrate along the trunks from a more cephalic level only a trunk with scattered ganglion cells will be formed. The embryos of group 1 thus provide convincing evidence that the sympathetic ganglia result from the migration of cells from the neural tube, but no evidence that they result from the migration of cells from the ends of the operated area.

Detwiler ('37) has suggested that the cells which are to form the sympathetic trunks may migrate from the neural crest at an early age before any differentiation of the spinal ganglion has occurred. If so, then it would be possible to extirpate the neural crest elements which are to form the spinal ganglia while leaving the elements that are to form the sympathetic ganglia. Indeed, Müller and Ingvar use this idea to explain the presence of sympathetic ganglion cells in an area from which the crests have been removed. Detwiler bases this hypothesis on evidence obtained from the vital staining of amphibian larvae. He finds that certain cells of the neural crest migrate ventrally into the region lateral to the aorta before any differentiation of spinal ganglia takes place. Due to their position he looks upon these cells as pre-sympathetic elements. However, the neural crests of *Amphibia* are known to be quite different from those of *amniotes*. The former give rise to connective tissue, corium and branchial cartilage as well as spinal ganglia. Accordingly, it is more probable that those cells which Detwiler regards as pre-sympathetic elements are merely the advance guard of tissue cells.

In any event, the situation demonstrates how dangerous it is, in this case, to draw deduction from amphibian experiments, especially when it is realized that all those who have studied the origin of the sympathetic system in the chick agree that the sympathetic elements migrate from the region at the ventral extremity of the spinal ganglion at about the close of the third day. The spinal ganglion is well differentiated at this time, and the cells which migrate from the region of its ventral border are neuroblasts, not undifferentiated cells. The time at which the migration takes place and the path which is followed are well known. The only question is whether the cells come from the ventral part of the spinal ganglion or from the neural tube.

Another line of evidence which has been presented in favor of the spinal ganglion hypothesis is that which is based upon the presence of sympathetic chains in amyelous monsters. Among others, Sherrington (1895) has described a 7-month fetus in which the brain and spinal cord were represented mainly by an irregular longitudinal membrane occupying the middle of a gutter formed by an open dura mater. Yet on the under side of the dura were complete rows of well-formed spinal ganglia, the distal roots of which were connected to normal sympathetic chains and to the usual set of peripheral nerves. Since no ventral roots were present, it would seem that the sympathetic trunks must have arisen exclusively from spinal ganglia. Fortunately, one of the chick embryos in our series (chick A) supplies additional evidence regarding the early stages of such amyelous forms. In this particular case the attempt to remove the neural crests miscarried but as a result of the operation the neural tube remained wide open for some distance. Nevertheless, spinal ganglia, ventral roots and sympathetic trunks formed as usual (fig. 14). But since the ependymal surface of this 7-day chick was everywhere exposed to the exterior, it is highly probable that this flattened ribbon of nervous tissue would have degenerated if the chick had been incubated longer.

In corroboration of this view one may cite a specimen of spina bifida contributed by the department of pathology. Spinal ganglia and sympathetic trunks were present, but the spinal cord consisted only of neuroglia. The presence of neuroglia indicated that nervous tissue must have originally been present. Degeneration of the nerve cells in the cord would, of course, result in degeneration of the ventral roots. It is apparent, therefore, that the persistence of sympathetic trunks in the absence of ventral roots, in amyelous fetuses, affords no proof that the sympathetic system is derived exclusively from spinal ganglia.

The conclusions reached in this work are directly opposed to those arrived at by Müller and Ingvar. The negative results obtained by these authors in performing experiments similar to ours are believed to be a direct consequence of their use of the electric cautery. Not only does this method fail to produce as clean-cut an operation as direct ablation with fine instruments, but also it damages surrounding tissues for an indeterminate distance. Since the development of the sympathetic system begins about the close of the third day and the embryos of Müller and Ingvar were fixed after a total of only 4 days of incubation, it is quite possible that cauterization of the region of the neural crests may have retarded the development of the nervous system to such an extent that no sympathetic elements had migrated out along the ventral roots before the embryos were killed.

The conclusions of Van Campenhout ('30) regarding the neural crest origin of the sympathetic system in amphibians has not been corroborated by the recent work of Raven ('37). The latter author has shown that in these lower forms cells which migrate from the ventral part of the neural tube help to form the sympathetic system.

No evidence is available in the present study regarding the contribution, if any, which the neural crest makes toward the development of the sympathetic system. All efforts by the author to use the Nile blue sulphate vital staining technique on the chick have failed. The fact that sympathetic trunks

will form following the removal of the neural crests does not necessarily prove that the crests contribute nothing to the normal sympathetic system. Some technique other than that so far employed must be utilized before this question can be answered.

In closing this discussion, it seems appropriate to mention the fact that the sympathetic system is an efferent system. Why, then, should it not arise from the efferent division of the nervous system?

CONCLUSIONS

Removal of the neural crest and contiguous areas of the neural tube in chick embryos that have been incubated for 40 to 48 hours, does not prevent the formation of sympathetic trunks in the area of operation although the corresponding spinal ganglia fail to develop.

The possibility that the sympathetic cells of such embryos might have come from the uninjured spinal ganglia at the ends of the area of operation is disproved by experiments which show that sympathetic cells will not migrate from the caudal end into an area where the neural tube has been removed, nor will they migrate more than three segments into the region from the cephalic end. Therefore, it seems logical to assume that the sympathetic ganglia which develop in the absence of as many as seven consecutive pairs of spinal ganglia must have migrated from the spinal cord along the ventral roots.

This, indeed, would seem to be the most natural source of sympathetic cells since the sympathetic system is an efferent system. Furthermore, examination of especially stained chick embryos which have been incubated 3 days—the time at which active migration of neuroblasts into the sympathetic path takes place—shows an unbroken continuity of cells of similar character from spinal cord to primary sympathetic chains. These differ in no way from the cells found in embryos of the same age from which the neural crests had been removed.

The negative results obtained by Müller and Ingvar ('23) who sought to prove that removal of the neural crest would prevent the formation of sympathetic ganglia, are explained on the ground that by employing an electric cautery, these investigators had injured more than the neural crests and so had delayed or prevented the outgrowth of neuroblasts from the ventral portion of the spinal cord.

Similarly, the significance attached to the occurrence of both spinal and sympathetic ganglia in amyelous monsters without ventral roots is discounted by an experiment in which it was possible to show that ventral roots are present in a 7-day chick in which an open neural tube represents the initial stage of amyelia.

The weight of evidence afforded by these experiments thus favors the view that the source of most, if not all, of the neuroblasts that form the sympathetic trunks, is the neural tube.

LITERATURE CITED

- CAJAL, S. RAMON 1890 À quelle époque apparaissent les expansions des la moëlle épinière du poulet. *Anat. Anz.*, Bd. 5, S. 609-613.
- DETWILER, S. R. 1937 Observations upon the migration of neural crest cells, and upon the development of the spinal ganglia and vertebral arches in *Amblystoma*. *Am. J. Anat.*, vol. 61, pp. 63-94.
- FRORIEP, A. 1907 Die Entwicklung und Bau des autonomen Nervensystems. *Medizin-Naturwiss. Arch.*, Bd. 1, S. 301-321.
- GANFINI, C. 1917 Lo sviluppo del sistema nervoso simpatico nei Mammiferi. *Arch. ital. d. anat. e d. embriol.*, T. 16, pp. 43-125.
- HIS, W., JR. 1897 Ueber die Entwicklung des Bauch sympathicus beim Hühnchen und Menschen. *Arch. f. Anat. u. Entw.*, Suppl., S. 137-170.
- KUNTZ, A. 1910 The development of the sympathetic nervous system in birds. *J. Comp. Neur.*, vol. 20, pp. 283-308.
- 1922 Experimental studies on the histogenesis of the sympathetic nervous system. *Ibid.*, vol. 34, pp. 1-26.
- 1926 The role of cells of medullary origin in the development of the sympathetic trunks. *Ibid.*, vol. 40, pp. 389-408.
- 1934 *Autonomic Nervous System*, pp. 111-112. Lea and Febiger, Philadelphia.
- MÜLLER, E., AND S. INGVAR 1923 Ueber den Ursprung des Sympathicus beim Hühnchen. *Arch. f. mikr. Anat.*, Bd. 99, S. 650-671.
- RAT, S., AND P. JOHNSON 1923 Observations on the development of the sympathetic nervous system and suprarenal bodies in the sparrow. *Proc. Zool. Soc. London*, vol. 2, pp. 741-768.
- RAVEN, CHR. P. 1937 Experiments on the origin of the sheath cells and sympathetic neuroblasts in *Amphibia*. *J. Comp. Neur.*, vol. 67, pp. 221-240.

- SHERRINGTON, C. S. 1895 On the anatomical constitution of nerves of skeletal muscles; with remarks on recurrent fibers in the ventral spinal nerve root. *J. Physiol.*, vol. 17, pp. 211-258.
- UCHIDA, S. 1927 Ueber die Entwicklung des sympathischen Nervensystem bei den Vögeln. *Acta. Schol. Med. Univ. Imp. Kioto*, Bd. 10, S. 63-94.
- VAN CAMPENHOUT, E. 1930 a Historical survey of the development of the sympathetic nervous system. *Quart. Rev. Biol.*, vol. 5, pp. 217-234.
- 1930 b Contribution to the problem of the development of the sympathetic nervous system. *J. Exp. Zool.*, vol. 56, pp. 295-320.
- 1931 Le développement du système nerveux sympathique chez le poulet. *Arch. de Biol.*, T. 42, pp. 479-506.

PLATE 1

EXPLANATION OF FIGURES

- 5 Section of 7-day chick in the thoracic region showing the normal relationship of spinal cord (s.c.), spinal (s.g.) and sympathetic ganglia (sy.) and ventral roots (v.).
- 6 Section of chick D at level indicated by arrow in figure 2. n, notochord.
- 7 Section of chick K at level indicated by arrow in figure 2. Note the absence of spinal ganglia.
- 8 High power view of spinal and sympathetic ganglia in a normal 7-day chick.
- 9 High power view of the ventral root of chick S shown at v. in figure 13.
- 10 Section of chick J at level indicated in figure 2.
- 11 Section of chick W (fig. 12) in region devoid of nervous tissue. Note that cartilages occupy the area where notochord and spinal cord should be.

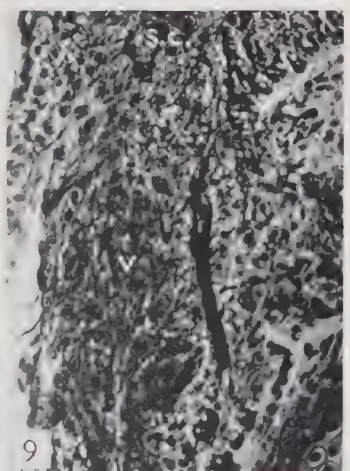
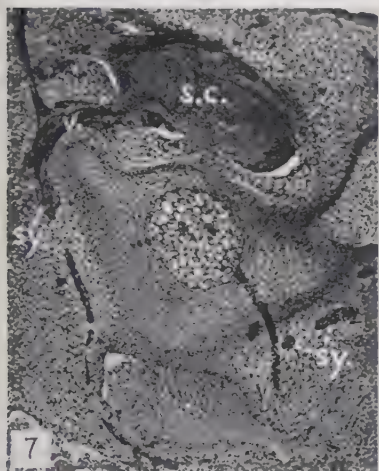
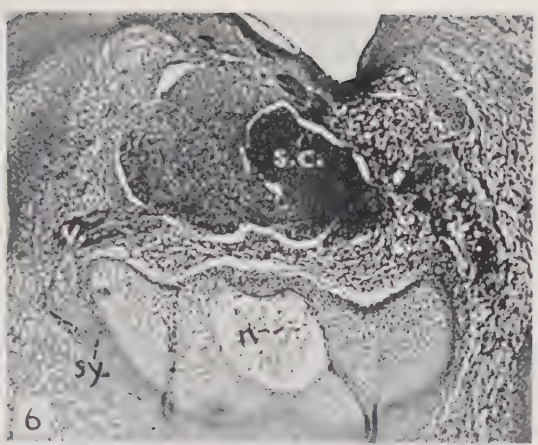
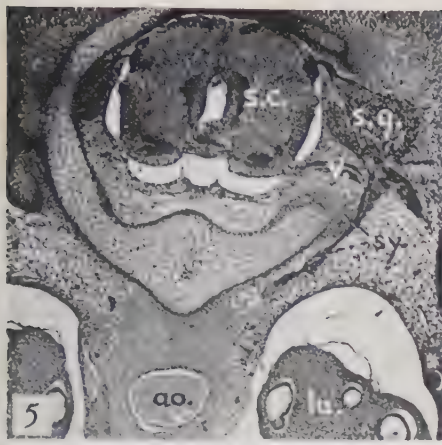


PLATE 2

EXPLANATION OF FIGURES

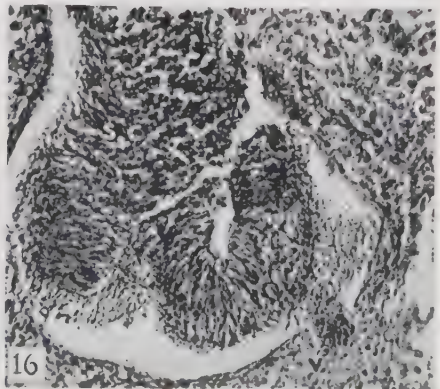
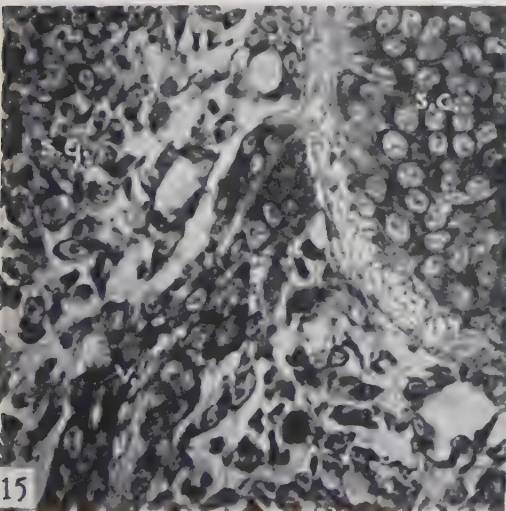
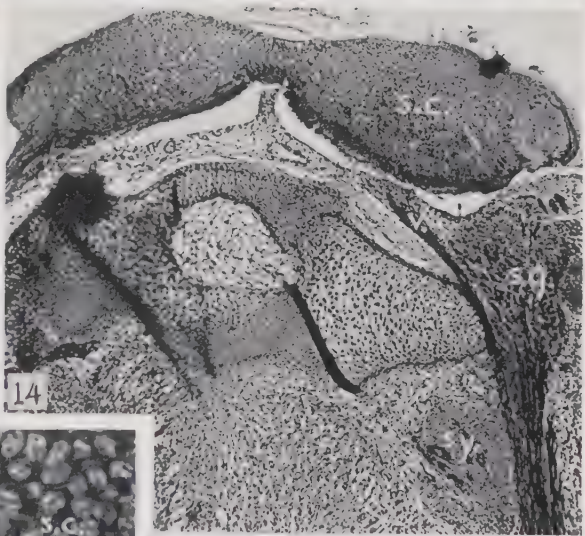
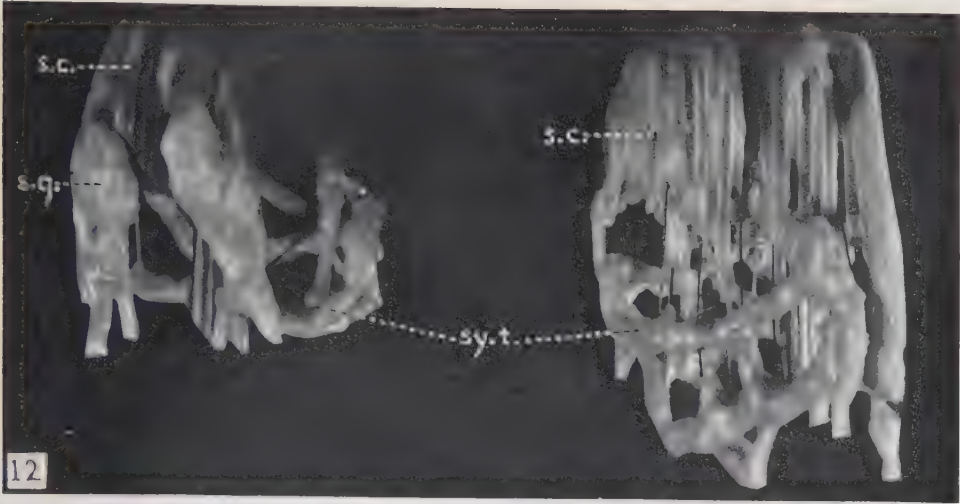
12 Wax reconstruction of chick W in the area of operation. sy.t., sympathetic trunks.

13 Section of chick S at level indicated by arrow in figure 4. Note continuity of cells of spinal cord (s.c.) and ventral root (v.).

14 Section of chick A showing open neural tube, ventral root and spinal and sympathetic ganglia. An incipient stage of amyelia.

15 Section of normal 3-day chick, showing continuity of cells within the ventral root and spinal cord.

16 Section of 3-day chick from which the neural crests were removed at 45 hours of incubation. Note cells in ventral root (v.).



A RARE CARDIAC ANOMALY

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ONE PLATE (THREE FIGURES)

The specimen to be described was the heart of a Negro baby, 7 months of age. The history which accompanied the specimen indicated a probable congenital cardiac malformation. Clinical symptoms were: severe attacks of dyspnea; cyanosis which became quite marked just before death; and progressive loss in weight. The autopsy findings were: an extremely enlarged right auricle; lungs pale and almost bloodless. No other pathology or anomaly was noted at the time of autopsy. The specimen was brought to this laboratory by Dr. B. F. Hauenstein of the Tompkins County Laboratory, Ithaca, N. Y., to whom the writer wishes to express his appreciation.

The gross specimen measured 29 mm. from base to apex and 36 mm. across the base. The enlarged right auricle measured $22 \times 30 \times 16$ mm. and was filled with a post mortem clot. The left auricle appeared quite normal in all respects and contained a small amount of blood. The right and left atria were also normal. There was a persistent left horn of the sinus venosus, and the coronary sinus had not yet been fully taken into the base of the heart. The ventricular surfaces of the heart were normal. The coronary arteries were quite tortuous throughout their course. The cardiac veins were well distended. The ascending aorta was enlarged and had a lumen of 9 mm. Its wall measured 1 mm. in thickness. The pulmonary arteries were atrophic; the right pulmonary was barely patent while the left had a lumen of only 2 mm. The

ductus arteriosus was almost completely obliterated. Figures 1 and 2 are ventral and left lateral views respectively of the gross specimen.

The heart was grossly sectioned, bisecting the aorta. The left ventricle was normal as was the mitral valve. The wall of the ventricle was hypertrophied measuring 8 mm. in thickness. The right ventricle, however, was represented by a mere slit which appeared to be a recess of the left ventricle and in communication with it just beneath the posterior semi-lunar valve. The right and left atria were normal save for the lack of an opening into the right ventricle from the right atrium. There was no indication of the existence of a tricuspid valve. The foramen ovale was widely open, the aperture measuring 9×6 mm. The pulmonary valve had merely two leaflets.

The primary defect in this specimen is the lack of a tricuspid valve. The persistent foramen ovale and the enlarged right auricle were dependent on this condition. Also, the gradual atrophy of the vessels leading from the right ventricle was a result of the reduction in blood flow through them due to the relative disadvantage of the route for the force of the current.

The main force of the flow would be directed through the aorta while only a small portion of it could be thrown backward through the small recess representing the right ventricle. It is also felt that the size of the ventricle is due to this failure to receive an adequate flow of blood either from the connection with the left ventricle or through the right atrium. From this poor development, it can be seen that the asymmetry is marked, but it is believed that the tissue labeled i.s. in figure 3 represents the interventricular septum.

Failure of the dorsal and ventral endocardial cushions to fuse in the customary 'figure of eight' fashion in the formation of the atrio-ventricular valves is interpreted as being responsible for the defect. The right side was completely fused, thereby obliterating the future tricuspid valve. This may have been due to some primitive asymmetry in the

heart itself whereby the flow of blood was not directed down along the sides in sufficient quantity or force to maintain the patency of the canal. The presence of only two pulmonary leaflets is due to atypical division of the bulbus.

The case is unique insofar as I have been able to ascertain in a search through the literature. Taussig ('36) in discussing the clinical findings in cases of tricuspid atresia or hypoplasia, states that true trilobulate conditions are rare. This case seems to represent that condition.

LITERATURE CITED

- TAUSSIG, H. B. 1936 The clinical and pathological findings in congenital malformations of the heart due to defective development of the right ventricle associated with tricuspid atresia or hypoplasia. *Bull. Johns Hopkins Hosp.*, vol. 59, pp. 435-444.

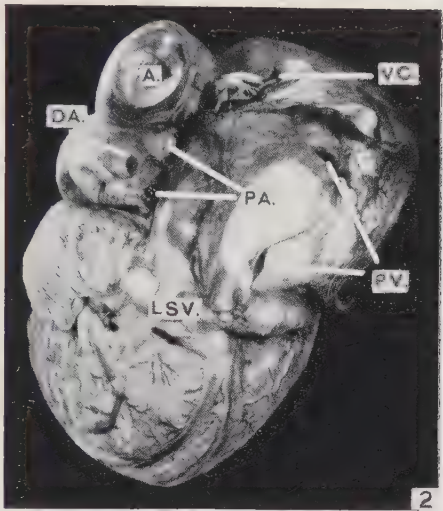
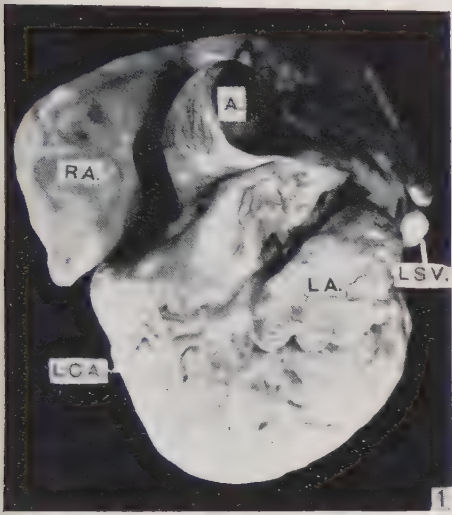
PLATE 1

EXPLANATION OF FIGURES

- 1 Ventral view of gross specimen. $\times 1\frac{1}{2}$.
- 2 Left lateral view of gross specimen. $\times 1\frac{1}{2}$.
- 3 Frontal section of gross specimen, bisecting the aorta. $\times 1\frac{1}{2}$.

ABBREVIATIONS

A., dorsal aorta	PA., pulmonary arteries
DA., ductus arteriosus	PV., pulmonary veins
LA., left auricle	RA., right auricle
LCA., left coronary artery	RV., right ventricle
LSV., left horn of sinus venosus	VC., superior vena cava
LV., left ventricle	i.s., interventricular septum



THE EFFECT OF FAT IN SIMPLIFIED DIETS ON THE REPRODUCTIVE ORGANS OF THE FEMALE ALBINO RAT DURING GESTATION

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ONE FIGURE

INTRODUCTION

Interest has recently been stimulated by the low-fat studies of Burr and Burr, and Evans and Lepkovsky, on the relationship between fat deficiency and disturbances in reproduction. The present investigation was undertaken to study in detail the effect of fat-poor diets upon the reproductive organs of the female albino rat during gestation. I wish to thank Dr. Jennings C. Litzenberg, Dr. C. M. Jackson, Dr. George O. Burr and Dr. W. R. Brown for their invaluable advice and criticism during the course of this investigation.

MATERIAL AND METHODS

Eighty-seven female albino rats (*Mus norvegicus albinus*) of the Minnesota-Wistar strain were used in the experiments. The females were bred from stock animals reared on McCollum's diet no. 1, and were placed on diet 551-B (Brown and Burr, '36) at weaning. The weaning weight averaged 44 gm.

Diet 551-B consists of:

- 84.1% sucrose (commercial)
- 12.0% crude casein
- 3.9% salt mixture (McCollum's no. 185).

¹ Accepted in partial fulfillment of the requirements for the degree of doctor of philosophy at University of Minnesota.

In addition, each animal was individually fed 0.65 gm. of ether extracted dry yeast, 44 γ of British Drug House 'A,' viosterol equal to 2 drops of high grade cod liver oil, and the non-saponifiable matter from 36 mg. of wheat germ oil. The rats were weighed weekly under carefully standardized conditions. At regular intervals following maturity, ovulation cycles were determined in order to classify the oestrous behavior of each animal.

When breeding was desired, the females at the pro-oestrous stage were placed in the cage of a tested male and allowed to remain over night. The first day of pregnancy was considered as the day following that on which the vaginal plug or spermatozoa were observed. Positive pregnancy was diagnosed by the appearance of 'red blood-cell sign' from the thirteenth to fifteenth day following insemination. Daily weights were recorded during gestation in order to detect resorbing pregnancies.

After a definite plateau in weight had been reached the animals were divided into two groups. The first group of forty-one animals was kept on a low-fat diet; the second group of sixteen animals was fed a supplement of fresh lard daily. These were then used later as the 'cured' controls. A third group of stock control animals (Minnesota-Wistar strain) was used as a comparative standard for the 'cured' control animals. A fourth small group of 'cured' control animals was later furnished from the low fat experiments of Brown and Burr. These animals differed from the second group in that the protein level was 24% crude casein and the lard dose 10 instead of 20 drops.

Animals from these groups were killed on successive days of pregnancy from the seventh to the twenty-second day. In the low-fat group, where prolonged gestation was observed, animals were sacrificed daily, up to and including the twenty-sixth day.

The preparation of material for microscopic examination consisted in the removal of the ovaries and the embryos or resorption bodies with a corresponding segment of the uterus.

These were fixed in Bouin's solution, cleared and dehydrated in dioxan solution and embedded in paraffin by the usual methods. The early specimens were sectioned and mounted serially; in the larger ones, every tenth section was mounted. Hematoxylin and eosin were used to stain all sections.

THE CHARACTERISTICS OF RATS REARED ON LOW-FAT DIETS

The clinical appearances and characteristics of such fat-poor animals conformed closely with those of Burr and Brown, whose animals were reared on the same diet in an adjacent colony room. The rats used in this investigation showed fat deficiency symptoms only to a moderate degree. The scaliness of the feet was not severe and the tail lesions were not the most marked. At autopsy marked renal lesions were not routinely found, and although the mesenteric fat was absent or very scant, the omental fat, as a rule, was abundant.

No indication of xerophthalmia occurred. The animals on the low-fat diet were not hyperactive and, although weighing only 80% as much as their controls, consumed about the same amount of food. It was found that the fat-poor animals consumed daily over two times the amount of water averaged by the controls. According to Burr and Burr ('30) the excess water consumption is evaporated from the lungs and skin.

The animals placed on a low-fat diet at weaning did not show any great retardation of growth during the first 60 days. As was previously reported by Burr and Burr ('29 and '30), the animals were always somewhat subnormal in size and reached a growth plateau earlier than normal. They started to plateau at about 4 months of age at a weight of approximately 156 to 158 gm. (fig. 1).

After being placed on a cure of 20 drops daily, or approximately 4% of fresh lard, which contains about 7% of the essential linolic acid, the deficiency symptoms started to disappear. In 3 or 4 weeks the scaly condition of the feet cleared up. The tail lesions disappeared, and there was a marked clearing of the skin. Dandruff disappeared and the hair coat became improved. Renewed growth both in length and weight (fig. 1) occurred almost immediately.

Burr and Burr ('30) observed that ovulation is often irregular or ceased entirely in fat-free animals (diet 550-B). When a curative oil is fed, ovulation is resumed. Female rats on the fat-free diet will mate when ovulation occurs. The added oils so improve the animals as a whole that ovulation is resumed. From the observation of 402 cycles of fifty-seven fat

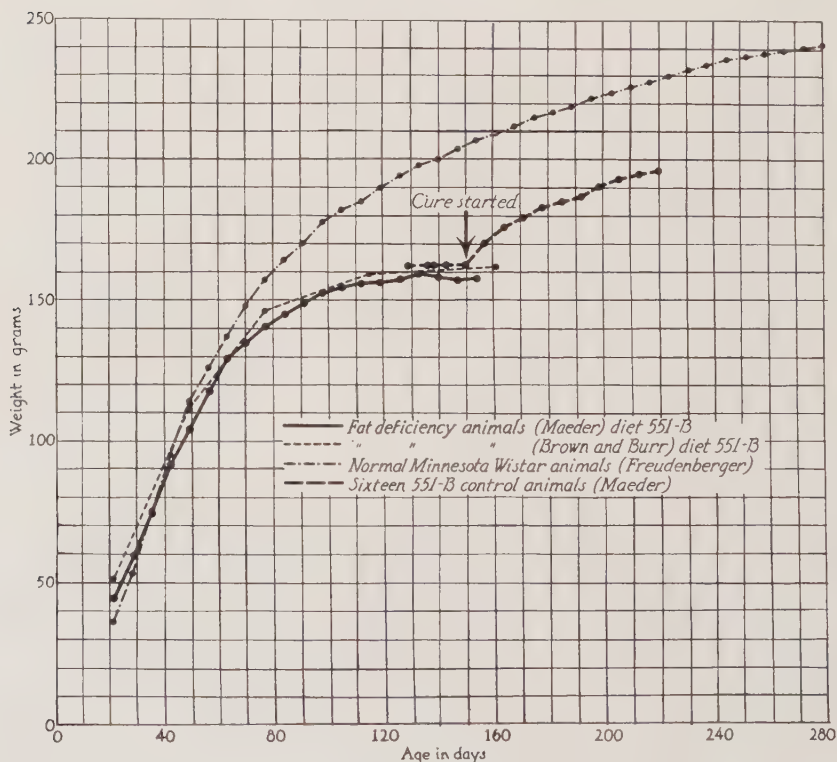


Fig. 1 Comparative growth curves of normal stock, fat deficient and fat 'cured' animals.

deficient animals, 80.35% were found to be of 3, 4 and 5 days duration. After sixteen animals from the above group had been on a cure for several weeks, 115 cycles were further studied, of which 96.52% were of the same duration.

Only three of the 'cured' control animals were allowed to litter. Parturition was normal; two of the litters were allowed to lactate and were subsequently successfully weaned.

GROSS CHANGES IN THE REPRODUCTIVE ORGANS OF FAT DEFICIENT RATS

The control 'cured' and normal stock animals revealed no essential difference in the gross nature of the reproductive organs and their contents.

In the pregnancies that failed to reach term, the abnormal enlargements were of a softer consistency and of a blue color, in marked contrast to the purple color of normal pregnancy. The blue discoloration was due to blood in the amniotic cavity. In the older resorptions the enlargements became more fusiform in shape and assumed a bluish-black color. When opened, they revealed only dark blood stained debris. Frequently copious hemorrhage into the lumen of the uterine horns was observed. The decidual bodies (Duval, 1891) at the mesometrial border were relatively prominent.

The great variation in the size of the uterine pregnancy sites indicated that the beginning or rate of resorption was by no means uniform. The conditions encountered were wholly unpredictable. Frequently dead and resorbing fetuses were intermingled with others whose gross appearance was normal. When all, or a portion of the fetal sites failed to undergo early resorption, growth of the fetus was often retarded and fetal death occurred at different periods during late pregnancy. Because in the fat deficient group of animals no incidence of successful parturition was encountered, it is believed that the normal sites observed at the different stages of pregnancy would have eventually succumbed either to resorption in utero or to late fetal death with or without delivery.

When resorption did not occur the pregnancies were frequently prolonged to the twenty-fourth, twenty-fifth and twenty-sixth days. If the resorption occurred late in pregnancy, or if there was prolongation of gestation, excessive amounts of dark brown, tarry-colored exudate with some necrotic cellular debris was usually observed in the vaginal smears, resulting in a delay in the reappearance of oestrus indicating some pathology of placentation. In a few cases of

early resorption blood was noted prematurely in the vaginal smears several days prior to the appearance of the red blood sign.

In the few animals that were delivered after prolonged gestation some difficulty in parturition with increased bleeding was noted but none of the animals died. However, during the period of prolonged gestation, the animals appeared weak, inactive and listless.

Periods of prolonged gestation usually terminated in the delivery of a variable number of dead and living fetuses. The litters were usually small and under-sized. The decrease in the average number of young per litter was due to a sporadic resorption of fetal sites during the early stages of pregnancy and also to an apparent relative failure of implantation. Growth of the fetuses in utero was quite variable. The live fetuses appeared weak and anemic, usually succumbing a few hours after delivery.

In the prolongation of gestation the placentas appeared dark and hemorrhagic. They were of soft consistency and varied in size. The amniotic fluid disappeared, in some instances, being represented only by a few strands of a stringy, tarry-colored material. In some cases the fetuses were cast inside with the membrane intact and with the degenerating placenta adherent outside. The fetuses in these cases were removed with difficulty from the dry amniotic sacs. No gross evidence of pus was noted. Hemorrhage and edema of the uterine wall were frequently observed suggestive of the 'Couvelaire uterus' seen in human cases of premature separation of the placenta.

The ovaries of the low-fat animals where resorption and prolonged gestation occurred showed characteristic gross changes. The corpora lutea of pregnancy appeared larger, paler and became chalky white in color. The ovaries showing these signs of degeneration weighed relatively more than those of the controls. No new or extra corpora lutea were observed in prolonged gestation.

MICROSCOPIC CHANGES

In some of the fat deficient animals, blood was observed early in the uterine lumen, soon after implantation had occurred. Free blood in the uterine lumen during early stages of pregnancy has been found by Huber ('15) in uteri possessing an abnormal endometrium. Evans, Burr and Althausen ('27) noted in pregnant E-free mothers on the eighth day of pregnancy extravasated blood in and about the free lumen of the uterus which lies mesometrial to the decidual cavity in which the egg is found.

The placenta gave a varied histological picture. Areas of localized hemorrhage from rupture of the maternal blood sinuses were commonly observed near the periphery of the placenta with exudation into the uterine cavity. Distension and rupture of the vascular sinuses of both the maternal decidua and fetal trophoblast were frequently seen, possibly due to an increased permeability of the vascular endothelium. Edema was often noted associated with the vascular congestion and hemorrhage in both the uterine wall and placenta.

Following this severe hemorrhage and decidual injury, areas of necrosis of varying intensity were observed, starting usually at the maternal side of the placenta and progressing toward the fetal surface. The maternal tissues were primarily involved more than the fetal structures. Areas of focal necrosis were occasionally observed in the decidua near the region of the trophoblast.

In cases of early resorption no definite evidence of infection was encountered. However, in the late resorptions and cases of prolonged gestation considerable evidence of inflammation, most likely secondary in nature, was observed. These areas of focal and diffuse exudation were associated with the process of necrosis. Both of these processes seemed to occur secondary to the hemorrhagic lesions. The histological picture was both that of an acute polymorphonuclear and polyblastic type of inflammation. In some sections only granulocytes and macrophages in varying proportions were observed, while in other sections a more low grade type of inflammation

with a predominance of lymphocytes and plasma cells was observed. The leukocytic infiltration was most common and intense usually at the junction of the placenta and chorion forming along with the associated necrosis, a ring around the periphery of the placenta. In many instances the leukocytic infiltration extended into the uterine wall.

In animals autopsied before the process of resorption had been initiated, normal anatomical development of both the placentas and fetuses was observed.

Except in cases of early resorption, the yolk sac with its entodermal villi, allantois, and other fetal structures showed normal development. In cases of prolonged gestation, the yolk sac structure could still be readily identified. Hematopoiesis and differentiation of fetal tissues revealed a normal histological picture. No failure of mesodermal elements was observed.

In spite of severe placental injury, very little retardation of fetal development was observed. Marked decidual and placental injury invariably preceded the alteration in the fetal tissues. The fetal injury consisted of general disintegration of the tissues due to lack of nutrition subsequent to placental injury. In late resorptions and prolonged gestation, where the disease was less acute, late fetal death and general retardation of fetal development was observed.

The intermuscular and mesometrial decidual reaction of the decidual body was delayed in the process of resorption, as compared with the control sections, still being visible at the end of pregnancy and in prolonged gestation. It regressed by lymphocytic and leukocytic invasion and often extravasation of blood was noted.

About the same time that abnormal histological findings were noted in the placenta and uterus, microscopic changes in the ovary were first observed. The luteal cells became coarsely granulated, clear and vacuolated; they seemed to lose their affinity for the eosin stain, and developed a foamy appearance. Vacuolization and coarse granulation of the luteal cell do not normally occur until about the seventeenth

day of pregnancy. By the end of pregnancy degeneration is moderately advanced, but many luteal cells retain their normal structure. No new corpora lutea or maturation of the graafian follicles were noted in the cases of prolonged gestation and late resorptions. Oestrus was considerably delayed in cases of prolonged gestation and late resorptions. Only in some instances of early resorptions did ovulation reappear before the end of the usual gestation period.

DISCUSSION

Rats used in this investigation and reared on diet 551-B developed typical fat deficiency symptoms. Brown and Burr ('36) stated that relatively impure diets may be used for the production of typical fat deficiency symptoms. When crude casein is substituted for purified casein, the weight at the time of plateau is slightly higher and decline and death are postponed. However, hematuria, scaly feet, and scaly and necrotic tails still occur to almost the same extent.

The process of reproduction was early impaired and was more resistant to treatment with curative doses of lard, while ovulation was affected late and returned to normal a few weeks after the animal received the lacking dietary unsaturated fatty acids. The dosage of the oil fed seemed to affect also the rate of cure. Two of the 'cured' animals (Maeder) after receiving 20 drops of lard for 95 days, had successful litters, while some of the Brown and Burr 'cured' rats, after receiving 10 drops of lard daily for over a year, failed to reproduce although manifesting external clinical cures. This phenomena is probably explained on the basis that physiological cures are not as rapid as external or clinical cures. Hansen and Brown ('37) have shown that animals given small quantities of the methyl esters of linolic acid sufficient to affect clinical cures were found to have a very little change in the iodine number of the total blood lipids. In general, once the physiology of reproduction was definitely interfered with, considerable difficulty was encountered in affecting an internal cure. In some cases the condition seemed irreparable.

Low-fat diets resulted in atrophic changes in the uterine mucosa and maternal decidua, interfering with the nutrition necessary for the satisfactory nidation of the fertilized ovum, and for the successful continuation and termination of gestation. A definite relative decrease in the number of implantation sites was apparent. These observations suggested some disorder of implantation, due most likely to a lack of hormonal stimuli, which interfered with uterine nutrition. If the fatty acids are associated with the ovarian hormones, it is possible that on a fat deficient diet the synthesis of ovarian hormone may be impaired.

When the fetal sites failed to undergo early resorption, fetal death occurred at different times during late pregnancy often resulting in prolonged gestation due to abnormalities of placentation which were frequently associated with copious hemorrhage. Variation in the size of the placenta with hemorrhage, tissue necrosis and infection in the placenta and uterine wall was often noted. These utero-placental changes are similar to these noted by Mason ('35) in vitamin 'A' deficiency and by Ueno ('34) where there was a lack of vitamin B.

In prolonged gestations there was a significant decrease in the average number of young per litter and a marked variation in the size of the dead and living fetuses delivered or observed at autopsy. Death of the fetus during the later stages of pregnancy was attributed to a less acute manifestation of the same factors responsible for earlier resorption. However, the small litters were usually undersized and the living young anemic, weak and of impaired vitality. According to King ('15) it is possible that prolongation of the gestation period for even 1 day materially increases the weight of the young at birth and further adds that individuals in small litters weigh more at birth than do individuals in large litters.

Prolonged gestation up to 26 days and difficult parturition was frequently associated with copious hemorrhage and great weakness of the mother. These findings agree with those of Evans and Lepkovsky ('34) and are similar to the observations of Mason ('35) on the prolongation of pregnancy and

the associated phenomena in vitamin A deficiency. No signs of xerophthalmia, abnormal vaginal cornification, or any other indication of vitamin A deficiency were observed. Similar prolongation of gestation was also noted by Barry ('20) in rats coming to term after underfeeding from the eleventh day of gestation. However, in view of the large proportion of deliveries observed by Barry ('20) in rats in which inanition was much more severe than in our fat deficient rats, there is no reason to believe that growth retardation played a significant role in the reproductive disturbances. On the other hand, Burr and Brown ('36) report that when fats high in unsaturated fatty acids are fed, the gestation period tends to be less than that found for normal stock animals. The prolongation of gestation and difficult parturition is apparently due in part to decreased vitality and death of fetuses during the late stages of pregnancy. Another contributing factor is the general weakness of the mother, associated with decreased tone of the abdominal musculature. Decreased responsiveness of the uterine musculature due to lack of necessary hormonal and mechanical stimulation is another important consideration. Furthermore, toxicity arising from the autolysis of the placental and fetal tissues must be recognized.

In the fat-deficient animals, secondary fetal death is best explained on the basis of decreased nutritive supply as a result of hemorrhage and tissue injury in the placenta and uterine wall. In vitamin A deficiency Mason ('35) writes that fetal death is also secondary to marked placental injury. There is no interference primarily with the development of the fetal structures and hematopoiesis as in vitamin E deficiency. According to Evans and Burr ('27) and Urner ('31), lack of vitamin E produces a retardation of development and differentiation of the fetal tissue, with no direct involvement of the maternal tissues.

It was particularly noted that in some of the partially 'cured' control group, which had first been bred before a physiological cure had been effected, that ovulation was considerably delayed following the process of late resorption. It

seems that postpartum oestrus is considerably delayed by the presence of retained intra-uterine gestational products. These same animals often showed bloody vaginal smears due to excessive uterine bleeding for many days following the expected date of delivery. Newton ('35) states that the placenta plays an essential part in the inhibition of oestrus during the latter half of pregnancy and probably also determines the date of parturition. After delivery of the placenta (pseudoparturition) oestrus occurs in 1 to 2 days.

Coincidentally with the placental change, alterations both grossly and microscopically were observed in the ovaries. In the fat deficient rat during pregnancy the degenerating ovaries became relatively larger and heavier than those found under normal pregnancy conditions. In cases of late pregnancy resorptions and prolonged gestation, the ovaries appeared pale, prominent and chalky. Evans and Lepkovsky ('34) noted that the ovaries of fat deficient rats in cases of prolonged gestation very frequently had an unnatural white, chalky appearance. Mason ('35) found in rats autopsied after prolonged gestations that the ovaries usually contained very pale and prominent corpora lutea. Microscopically, these large chalky corpora lutea revealed degenerative changes in the luteal cells which become clear and vacuolized, presenting a foamy appearance. Some of the cells besides losing their affinity for the eosin stain, become coarsely granulated. This histo-pathological picture corresponds to that observed by Kasper ('31) in vitamin E deficient rats.

Mason ('35) removed the ovaries of vitamin A deficient rats between the eighteenth and twenty-first days of pregnancy and failed to demonstrate any lessening of the abnormalities of late gestation in the operated rats, thus ruling out hyperfunction of the corpora lutea as the cause of prolonged gestation. Prolongation of gestation and abnormalities of parturition have been observed by Snyder ('34) in rabbits in which an additional set of corpora lutea was induced by injection of extracts of pregnancy urine (Antuitrin S) near term. A normal number of corpora lutea was observed in the low-fat

animals both grossly and microscopically. The ova and developing graafian follicles revealed no histo-pathology.

These degenerative changes both in the ovaries and placenta appear to occur concomitantly, apparently resulting from the same etiologic basis, probably due to a lack of proper ovarian hormone synthesis and improper endocrine balance.

From extensive experimentation with rats, Selye, Collip and Thomson ('35) conclude that the placenta furnishes the necessary stimulus for the maintenance of the corpus luteum of pregnancy and determines the length of pregnancy either by its effect on the corpus luteum or by its own progesterin production, or which is more likely, by both of these means. They further found that in the rat ovariectomy during pregnancy does not interfere with the life of the placenta. It terminates pregnancy only because it causes the death of the fetus probably due to partial involution of the uterus which considerably increases the pressure in the gestation sac. The life span of the placenta was not markedly influenced by the removal of the embryo. The length of the gestation period must be determined by factors inherent in the placenta.

The decidual body appeared more prominent both grossly and microscopically in the pregnant fat deficient animals. Urner ('29) noted the same findings in the failed pregnancies of vitamin E deficient animals.

As a practical aspect of the study it might be well to point out at this time that as Burr and Burr ('30) suggest, the human diet is often exceedingly low in fats of any kind and that when fats are added they usually contain little of the acids more unsaturated than oleic. It is possible that our high carbohydrate and protein diets, carrying very little of the unsaturated oils, are contributory factors to poor health and sterility. The addition of egg yolk and cod liver oil to diets may often improve the patient because of the fatty acid rather than the vitamin content. For example, cures of anemia with cod liver oil have been reported and it has been shown that there is a relation between experimented anemia and unsaturated fatty acids of the blood plasma. The prevalence of dry

skin and abnormal kidneys may be directly attributed to improper fat intake. As the liver is apparently limited in its ability to produce these acids, they should be plentifully supplied through the diets.

SUMMARY

1. Animals reared on diet no. 551-B showed the typical characteristics of the fat deficiency syndrome.

2. Reproductive function is early impaired on a fat deficient diet. The findings substantiated the observations of Burr and Brown, that reproduction was not normal until some time after a clinical cure had been obtained.

3. Ovulation is impaired late on a fat-poor regimen, but responds early to curative doses of unsaturated fatty acids.

4. A definite relative failure of implantation was observed, due to atrophic changes and undevelopment of the uterine mucosa and maternal decidua.

5. Resorptions or prolonged gestation resulted from fat deficient diets. No successful gestations were obtained with fat deficient animals.

6. Histologically, variable degrees of hemorrhage, necrosis and secondary inflammatory exudate were observed in the placenta and uterine wall.

7. In fat deficiency, fetal death was secondary to marked placental injury.

8. Early interruption of gestation frequently occurred if the fat deficiency symptoms were very acute.

9. In less acute fat deficiency, prolonged gestation occurred.

10. Prolonged gestations were frequently associated with excessive uterine bleeding, difficult parturition and great weakness of the mother.

11. Lessened vitality or death of fetuses, decreased tone of the abdominal and uterine musculature, and possible lack of the necessary hormonal stimulation, were regarded as the important etiologic factors of prolonged gestation.

12. Degenerative changes in the ovaries of fat deficient animals occurred concomitantly with the utero-placental pathology. The large chalky ovaries, commonly observed, microscopically showed vacuolization and coarse granulation.

13. The decidual bodies are prominent in prolonged gestation and late resorption.

14. The failure of reproduction in humans due to lack of adequate fat in the diet is considered possible.

LITERATURE CITED

- BARRY, L. W. 1920 The effect of inanition in the albino rat with special reference to the changes in the relative weights of the various parts, systems and organs of the offspring. *Contrib. to Embryol.*, vol. 12, pp. 91-136.
- BROWN, W. R., AND G. O. BURE 1936 a Some recent studies in fat deficiency. *J. Biol. Chem., Proc. Am. Soc. Biol. Chem.*, vol. 114, p. 16.
- 1936 b Some effects of quality of fats on their nutritional value. Paper read at Kansas City before the Biological Section of the American Chemical Society.
- BURE, G. O., AND M. M. BURE 1929 A new deficiency disease produced by rigid exclusion of fat from diet. *J. Biol. Chem.*, vol. 62, pp. 345-367.
- 1930 On the nature and role of fatty acids essential in nutrition. *J. Biol. Chem.*, vol. 861, pp. 587-621.
- BURE, G. O., AND W. R. BROWN 1933 On fatty acids essential in nutrition. *Proc. Soc. Exp. Biol. and Med.*, vol. 30, pp. 1349-1352.
- DUVAL, M. 1889-1892 *Le Placenta des Rongeurs. J. de l'anat. et de la Physiol.*, T. 29-30.
- EVANS, H. M., G. O. BURE AND T. ALTHAUSEN 1927 The antisterility vitamin fat soluble E. *Memoirs of the University of Calif.*, vol. 8.
- 1927 New dietary deficiency with highly purified diets. II. Supplementary requirements of diet of pure casein, sucrose and salt. *Proc. Soc. Exp. Biol. and Med.*, vol. 25, pp. 41-48.
- 1928 A new dietary deficiency with highly purified diets. III. The beneficial effect of fat in diet. *Proc. Soc. Exp. Biol. and Med.*, vol. 25, pp. 390-397.
- EVANS, H. M., AND S. LEPKOVSKY 1932 Vital need of the body for certain unsaturated fatty acids. *J. Biol. Chem.*, vol. 96, pp. 143-155.
- 1934 Vital need of the body for certain unsaturated fatty acids. I. Reproduction and lactation upon fat-free diets. *J. Biol. Chem.*, vol. 106, pp. 431-440.
- HANSEN, A. E., AND W. R. BROWN 1937 Effect of dietary fats upon serum lipids of rats. *J. Nutrition*, vol. 13, pp. 351-357.
- HUBER, G. C. 1915 The development of the albino rat (*Mus norvegicus albinus*). *J. Morph.*, vol. 25, pp. 247-358.
- KASPER, E. M. 1931 The genital tract of the rat, with special reference to changes in pregnancy and during vitamin E deficiency. Thesis, Graduate School of the Univ. of Minn.

- KING, H. D. 1915 On the weight of the albino rat at birth and the factors that influence it. *Anat. Rec.*, vol. 9, pp. 213-231.
- MASON, K. E. 1935 Foetal death, prolonged gestation, and difficult parturition in the rat as a result of vitamin A deficiency. *Am. J. Anat.*, vol. 57, pp. 303-349.
- NEWTON, W. H. 1935 'Pseudo-parturition' in the mouse, and the relation of the placenta to post-partum oestrus. *J. Physiol.*, vol. 84, pp. 196-207.
- SELYE, H., J. B. COLLIP AND D. L. THOMSON 1935 Endocrine interrelations during pregnancy. *Endocrinology*, vol. 19, pp. 151-159.
- SNYDER, F. F. 1934 The prolongation of pregnancy and complications of parturition in rabbit following induction of ovulation near term. *Johns Hopkins Hosp. Bull.*, vol. 54, pp. 1-23.
- UENO, J. 1934 Experimental study of the effect of vitamin B in the female genital organs. *Jap. J. Obstet. and Gyn.*, vol. 17, p. 388.
- URNER, J. A. 1931 The intra-uterine changes in the pregnant albino rat (*Mus norvegicus*) deprived of vitamin E. *Anat. Rec.*, vol. 50, pp. 175-187.

EXPERIMENTALLY PRODUCED STERILE GONADS AND THE PROBLEM OF THE ORIGIN OF GERM CELLS IN THE CHICK EMBRYO

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FIVE FIGURES

The present study is an outgrowth of experiments primarily designed to test the validity of Swift's theory of the extra-gonadal origin of the germ cells in the chick. In the original experiments whole blastoderms, mostly of the head-process stage (about 19 hours incubation), were grown in chorio-allantoic grafts to ascertain their capacity to differentiate a gonad following the excision of the crescentic area of the so-called primordial germ cells (Willier, '26). It was found that gonad and other trunk organs failed to develop, anterior organs only being formed. Since in these experiments there appeared to be a causal connection between the position of the primitive knot and capacity to form embryonic parts, experiments were designed to analyze such a relationship (Hunt, '31; Willier and Rawles, '31) and as a consequence methods of obtaining gonads free of germ cells were discovered. By transplanting entire blastoderms of stages later than the head process, i.e., of 3 and 9 somites, a gonad free of germ cells was obtained for the first time (Willier, '32, p. 131). A similar result was obtained by transplanting the region containing the primitive knot of a blastoderm of the head-process stage. Subsequently a rather thorough study has been made using these two basic methods of analysis or modifications thereof.

¹ It is a pleasure to acknowledge the excellent assistance of Dr. Mary E. Rawles in this investigation.

In all cases the gonad which differentiates in such grafts is sterile and consists of typical male-like sex cords, ovarian cortex never having formed. This singular result not only presents a problem of considerable significance in itself but indicates that gonad origin and to a certain extent its differentiation, is independent of the so-called primordial germ cells. These problems together with a consideration of their bearing on the theory of the extra-embryonic origin of germ cells in the chick furnish the chief topics for analysis in this paper.

A preliminary report of the principal generalizations of the studies made on experimentally produced sterile gonads has been made briefly elsewhere (Willier, '32; Willier and Rawles, '36).

EXPERIMENTAL PROCEDURE

By means of the chorio-allantoic graft chick blastoderms at stages ranging from the head process (ca. 18 hours incubation) to those having 25 somites were analyzed for their capacity to form a gonad. Such a range includes a number of stages in the developmental history of the primordial germ cells. Large amoeboid cells, which were first described by Dantschakoff ('08) as 'entodermallen Wanderzellen' and subsequently identified by Swift ('14) as 'primordial germ cells,' arise from the germ-wall entoderm just at the antero-lateral margin of the pellucid area, the so-called 'germ cell crescent' (figs. 1, 2 and 3). They first make their appearance at the primitive streak stage and continue to form until the embryo has several somites. Thus they accumulate at the site of origin in groups in the space between the entoderm and ectoderm (fig. 3). With the ingrowth of mesoderm into this region the germ cells enter it, a few being found therein in 6- and 9-somite embryos. As blood vessels develop within the mesoderm, they are carried (or move by their own activity) to every part of the vascular area and embryo. This is the situation beginning with the 10- and 12-somite stages. They remain thus distributed until about the 22-somite stage, at which time they have entirely disappeared

(Dantschakoff). According to Swift all the germ cells are to be found in the blood vessels until the 21-somite stage, after which they gradually decrease in numbers until finally after the 25-somite stage none is found. During this period they are entering and accumulating in the tissues of the embryo, especially in the splanchnic mesoderm of the future gonad.

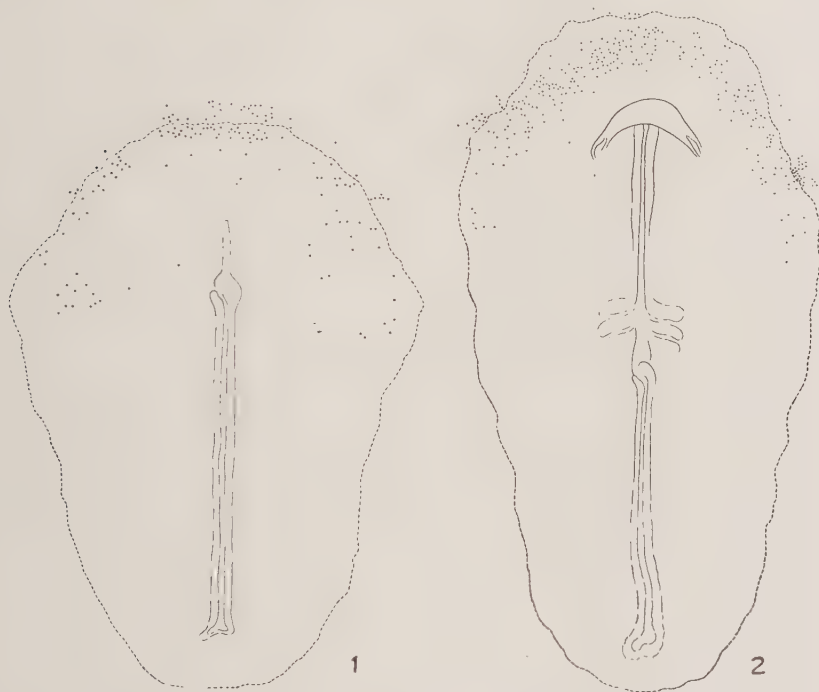


Fig. 1 Map showing distribution of the primordial germ cells in a head-process blastoderm. $\times 29$.

Fig. 2 Map showing distribution of the primordial germ cells in a 3-somite blastoderm. $\times 27$.

Summarily stated, we may recognize for purposes of the present experimental analysis three critical stages in the developmental history of the germ cells: blastoderms in which the germ cells 1) are at the site of origin 2) are in the blood vessels and 3) have entered the splanchnic mesoderm of the future gonad.

The manner of isolation of the portion of the blastoderm to be grafted is somewhat variable. In the majority of cases examined the transplant consisted of the entire pellucid area or the greater portion of it isolated in various ways. First, the transplant included all of the pellucid area except a narrow marginal zone, the line of excision falling just inside the junction of the opaque and pellucid areas. It was originally thought that such an isolation would free the transplant of all primordial germ cells. It is now apparent after a careful study of their distribution that while the locus of origin as well as the greater portion of them are discarded with the opaque area some undoubtedly remain in the pellucid area (fig. 1). This is generally true for the head-process blastoderm, the stage used in this type of isolation. In some cases particularly at later stages the germ cells are distributed in such a manner, viz., (as in fig. 2) that the majority would be located in the isolated pellucid area rather than in the discarded portion of the blastoderm. A second type of isolation was made so that all of the germ cells would be included in the pellucid area. In this case the line of excision falls external to the junction of the pellucid and opaque areas. The stages used varied from head process to 11-somites. A third type of isolation included all of the pellucid area caudal to the level just anterior to the heart primordium. As in the first case the line of excision of the portion grafted lay just inside the junction of the pellucid and opaque areas. The donor blastoderms ranged from 3- to 13-somite stages. A fourth type of implant was isolated so as to include all of the pellucid area between the level of either the twelfth or fourteenth pair of somites and the node level (but not including it). The donor embryos ranged from 12 to 25 somite stages, during which period the germ cells are supposedly in the blood circulation or are in the process of entering the tissues of the embryo.

In a smaller group of experiments the transplant included a small piece of blastoderm of the head-process stage containing the primitive knot of Hensen. Such pieces as tested measured approximately 0.3 mm. square.

In still another set of experiments the urino-genital ridges were isolated from donors having 29 to 41 somites. At these stages the germ cells are visible in the gonad-forming areas. Since these grafts were considered in a former report (Willier, '33, fig. 14, p. 641) they are included here for comparative purposes only.

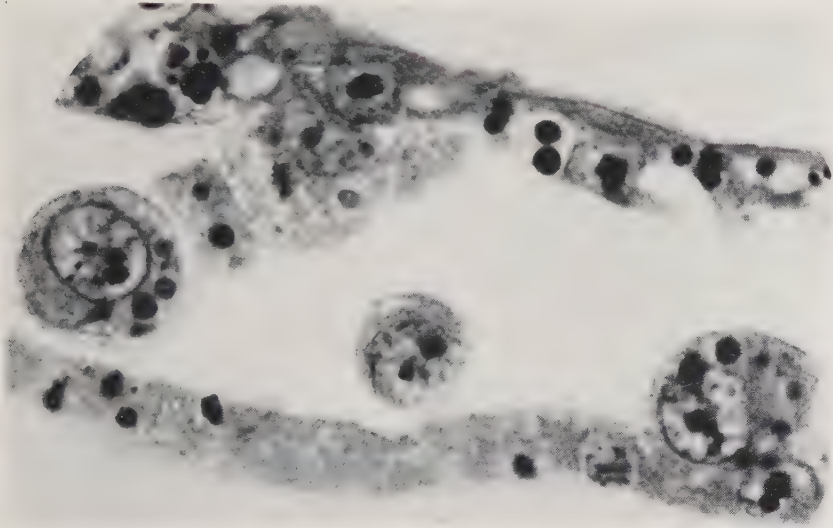


Fig. 3 Photograph of primordial germ cells situated between the ectoderm and entoderm of a 3-somite blastoderm. $\times 1500$.

DESCRIPTION OF THE RESULTS

1. *Entire pellucid area with most of the germ cell crescent excised.* Thirteen grafts, all from blastoderms of the head-process stage, were examined. They were large grafts containing usually all parts of the embryo back to and in two cases including the mesonephros. Neither gonad, adrenal nor metanephros differentiated. Germ cells were not observed (Willier and Rawles, '31).

2. *Entire pellucid area including the entire germ cell crescent.* From this type of transplant fourteen grafts were examined, two from blastoderms of the head-process stage and twelve of the somite stages (one each from 2-, 3-, 4- and

5-somite donors; two from 7-somite, three from 9-, one each from 9-, 10- and 11-somite donors). These grafts were similar to those described above in size and in parts of the embryo formed. Mesonephros but not metanephros has developed in six grafts, all from blastoderms of somite stages. Both adrenal and gonad are associated with the mesonephros in two cases (3- and 9-somite donors) and adrenal only in another case (7-somite donor).

Structure of gonad from 3-somite donor. This gonad is a tiny body lying next to the middle portion of a somewhat attenuated adrenal body. Both gonad and adrenal protrude into a coelom-like space in the mesenchyme of the graft. When the adrenal is traced through the sections it becomes more or less continuous with a much drawn-out body of mesonephric tissue. Several germ cells occur in the mesenchyme of this graft.

Structurally the gonad is composed of a very few male-like sexual cords each circumscribed by a thin connective tissue envelope. The cords are short and unbranched and consist of non-germinal cells somewhat irregularly arranged as a rule, but in three cords their nuclei occupy a more regular position next to the wall of the sex cord, resembling somewhat closely a normal testicular cord of a corresponding age except for the absence of germ cells. The amount of intercordanal connective tissue is small.

Gonad from 9-somite donor. This is the first sterile gonad ever obtained in our study. It is a cylindrical body, measuring $386\ \mu$ in length and $290\ \mu$ in diameter. It is surrounded with a coelom-like cavity, except on one side where it is continuous with the graft mesenchyme, and at one end where it joins the mesonephros. Lying in the mesenchyme near its union with the mesonephros is a mass of suprarenal tissue.

Like the gonad described above, it consists of male-like sexual cords, some intercordanal connective tissue and a tunic-like envelope of mesenchyme. In the intercordanal tissue there is a considerable number of blood spaces containing red blood corpuscles. The sexual cords range in form from irregular

collections of non-germinal cells to definitely circumscribed cords which are somewhat elongated and even branched (Willier, '32, p. 131, fig. 16). In some of the highly differentiated sex cords the nuclei of the non-germinal cells show a tendency to be arranged at the periphery of the cord as in the normal. More atypically, however, they are irregularly arranged, a condition which is characteristic of young sex cords. After a careful search no germ cells were recognized either in the sex cords, or in any other part of the gonad, or even elsewhere in the graft.

3. *Entire pellucid area except portion anterior to heart level.* Twenty grafts of this category were examined from donors having from 3 to 13 somites (one each from 3- and 4-somite, two from 5-somite, three from 6-somite, two from 7-somite, one from 8-somite, five from 9-somite, two from 10-somite, and one each from 11-, 12- and 13-somite embryos).

In this type of graft where the head region as well as the germ cell crescent were discarded, it was thought that if the size of the implant or the dominant head region were reduced, growth and differentiation of trunk parts might be favored, resulting in an increased frequency of gonad formation. Evidence indicates that this is the case, since mesonephros and adrenal have appeared with far greater frequency, the former in sixteen and the latter in thirteen grafts. Furthermore, a more posterior organ, the metanephros, has appeared definitely in two grafts and probably is present in two others. This organ occurs only in grafts containing both mesonephros and adrenal. With the few exceptions noted these grafts are similar in the kinds of parts differentiated to those described above. A small gonad associated with the mesonephros has developed in two grafts (6- and 10-somite donors). Metanephros and adrenal are also present in each of these grafts.

Gonad from 6-somite donor. In this graft, grown on a female host, two separate adreno-gonad-mesonephros complexes have developed very much as in the normal embryo. To one side (apparently dorsal) of them is a rather typical spinal cord surrounded by cartilaginous vertebrae. On the

opposite side (ventral) there are two coelom-like cavities in the mesenchyme each partially surrounding a mesonephros. Into these cavities two small gonads are suspended from each mesonephros. Adjacent to each mesonephros on the side toward the spinal cord is a mass of adrenal tissue. Each gonad is a definitely circumscribed cylindrical body of small dimensions (the one shown in fig. 4 measures $156\ \mu$ in length by $145\ \mu$ in diameter; the other three have similar dimensions). The two gonads of each mesonephric complex are separated by a gonad-free zone which apparently marks the line of constriction of an originally single gonad-forming area.

Histologically each gonad consists of three kinds of tissues, viz., male-like sexual cords, intercordal tissue, and next to the coelom-like space a tunic of connective tissue. The intercordal tissue is a stroma of connective tissue containing blood capillaries and occasionally a rounded cell. The sexual cords although relatively few in number are well-formed structures, only occasionally showing any indication of convolutions, or branches. They are made up of non-germinal cells only. As seen in figure 4 the sexual cords show a high degree of differentiation. The nuclei of the non-germinal cells are situated in a row at the periphery of the cord and from them cytoplasmic strands extend inward. This histological picture is similar to that seen in the sex cords of a normal testis of an embryo of the corresponding age (i.e., 12 days). In some of the cords the nuclei are less definitely arranged.

Gonad from 10-somite donor. The gonad in this case is a small cylindrical body about $198\ \mu$ in length and $146\ \mu$ in diameter, resembling in form and size the gonad just described. It, together with a mass of adrenal tissue in juxtaposition, is situated at one end of a much elongated mesonephros. The mesonephros and adrenal lie embedded in mesenchyme, while the gonad is surrounded, except where it unites with the mesonephros, by a coelom-like cavity. At the opposite end of the mesonephros lies a metanephric body.

With respect to the kinds of tissues, this gonad is very similar to the one just described. The tunica albuginea is

thinner and the sexual cords fewer and of smaller diameter. Some are definitely branched and the nuclei of the non-germinal cell are only occasionally typically situated at the periphery of certain cords.

4. *Portion of the pellucid area from approximately the 15-somite level to node level* (donors 12 to 25 somites). Twenty-four grafts have been examined from the level of the

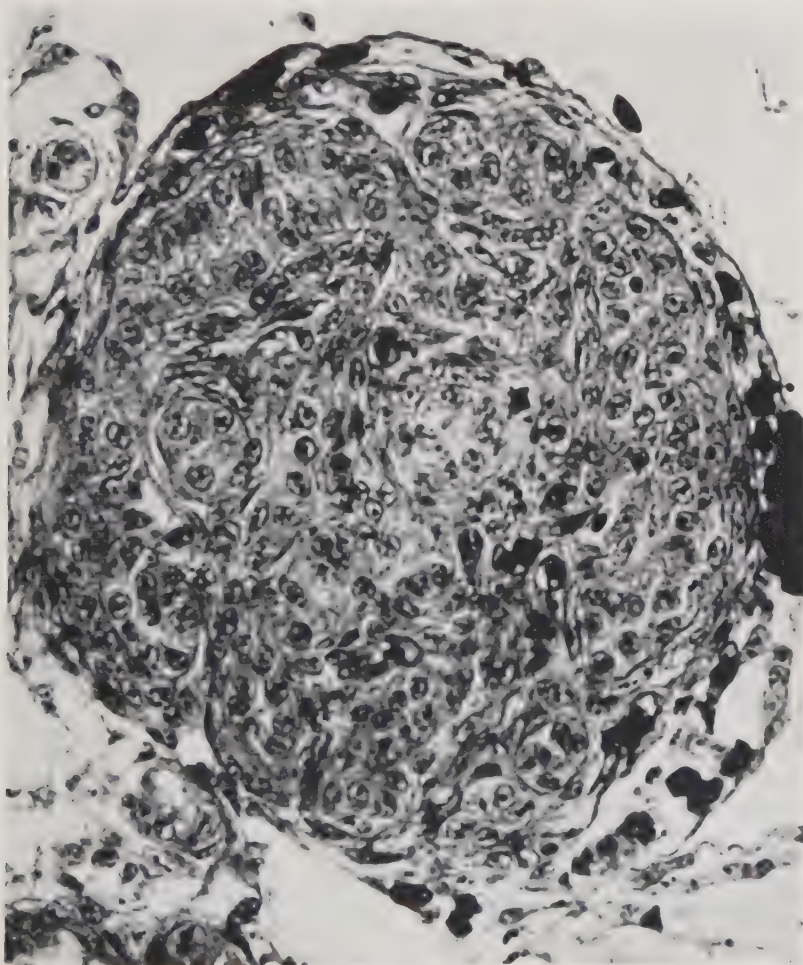


Fig. 4 A sterile gonad differentiated on a female host from a 6-somite blastoderm following the removal of the head and the entire germ cell crescent. $\times 650$.

pellucid area destined to form the mesonephros (from fifteenth to thirtieth somite in the normal embryo). The anterior margin of the transplant lay between the fourteenth and fifteenth somite level except in the 12- and 13-somite donors where it was between the last two somites formed. In all cases the posterior margin lay just in front of the node. Of the grafts examined, one came from a 12-somite, one from a 13-somite, two from 15-somite, three from 16-somite, two from 17-somite, two from 18-somite, two from 20-somite, two from 21-somite, one from 22-somite, two from 23-somite, four from 24-somite and two from 25-somite donors. The typical structures found in these grafts are spinal cord, ganglia, notochord, mesonephros and such generally-occurring tissues as gut, cartilage, bone, muscle, skin and feather germs.

Since we are dealing here with the level of the pellucid area normally concerned with the formation of the adrenal-gonad-mesonephros complex these tissues would be expected to occur with a high frequency. The results, however, turned out to be rather disappointing in this respect, since only fourteen of the twenty-four grafts showed any development of the mesonephros, and only about half of these showed adrenal. Gonad, appearing much less frequently than either mesonephros or adrenal, has differentiated in juxtaposition to these tissues in two cases as a small body devoid of germ cells. These two gonads, obtained from 15- and 18-somite donors respectively will now be described.

Gonad from 15-somite donor. The gonad in this case is an exceptionally small body ($120\ \mu \times 160\ \mu$). One side of it is continuous with a small mesonephros, while the other protrudes into a coelom-like cavity. Nearby in the mesenchyme is a small mass of adrenal cords. The free surface of the gonad is covered with a thin mesenchymal wall.

The sexual cords are quite few in number, short and slender, and in some cases not very distinctly outlined. In several cords the nuclei of the non-germinal cells are arranged at the surface of the cord, thus presenting a somewhat typical picture of a male sex cord.

Gonad from 18-somite donor. This gonad is a spherical body ($200\ \mu$ in diameter) situated at one end of a somewhat elongated mesonephros, where it protrudes into a large coelom-like cavity. Adrenal tissue is not associated with the gonad but with one or two other masses of mesonephric tissue found elsewhere in the same graft.

The gonad is made up of sterile sex cords ranging from a few which are typically male-like in structure (peripheral position of nuclei of non-germinal cells) to many which appear in section as nests of two or more non-germinal cells. Between the sex cords is an abundance of loose connective tissue containing numerous blood channels. Next to the 'coelome' the gonad is covered with a single layer of flattened cells. Between this and the sex cords is a comparatively thick layer of loose mesenchyme.

5. Piece of blastoderm containing the primitive knot. Fifteen grafts of the node region of the blastoderm in the head-process stage have been examined histologically. Mesonephros has differentiated in eight of these. Associated with it in two grafts are adrenal, gonad and metanephric tissues and in two others adrenal tissue only.

Gonad 223-5-1. In juxtaposition to one end of an elongated mass of mesonephric tissue lies a cylindrical gonad body of small dimensions ($175\ \mu \times 117\ \mu$). Adrenal does not appear in this particular complex but is connected with a second mass of mesonephric tissue found elsewhere in the graft. The gonad, although a definitely circumscribed body, is neither surrounded by a coelom nor covered with a tunica albuginea. Its surface is continuous with the surrounding loose mesenchyme except where it comes in contact with mesonephric tubules. It is made up of sex cords containing only non-germinal cells as may be seen in figure 5. The cords exhibit some variation in structure. A few are well defined with the nuclei of non-germinal cells peripherally situated and oriented. Many slender cords are found in which the non-germinal cells and their nuclei are irregularly arranged. Both types of cords may be branched or slightly convoluted.

Masses of non-germinal cells not formed into definite cords also occur.

Gonad 226-4-1. About mid position of an elongated mass of mesonephric tissue and in contact with it is found a gonad

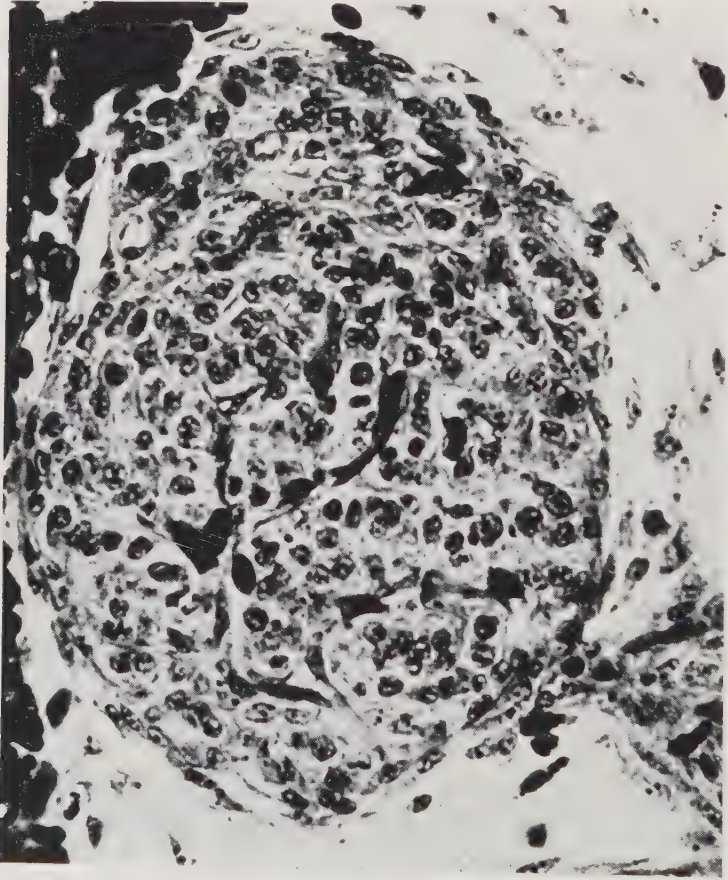


Fig. 5 A sterile gonad differentiated on a male host from a small portion containing Hensen's node of a head-process blastoderm. $\times 500$.

of small dimensions ($168\mu \times 117\mu$). At the surface of the mesonephros, about 90° away appears a small adrenal of poorly differentiated cords. This gonad is a definitely circumscribed cylindrical body lying in loose mesenchyme. In juxtaposition are two slender extensions of coelom-like spaces, one

of which actually comes in contact with the sex cords of the gonad. Structurally the sex cords are very similar to those just described; in number, however, they are fewer.

DISCUSSION

Extra-gonadal origin of the germ cells. The development of a gonad free of germ cells as found and described in the present series of grafting experiments furnishes strong evidence that 'the germ cell crescent' of Swift is the source of the germ cells of the gonad rudiment. This result is in agreement with the findings of other investigators who used different methods of freeing blastoderms of primordial germ cells in early stages of development (just prior to formation of first somite). The 'germ cell crescent' of the blastoderm has been excised (Reagan, '16), irradiated with ultraviolet (Benoit, '30) and destroyed by means of electric cautery (Dantschakoff et al., '31). A histological examination of blastoderms thus treated and incubated for 2 or more days reveals the entire absence of germ cells from the gonad-forming areas. It is thus seen that regardless of the method used for removing germ cells from the blastoderm the gonad-forming area which subsequently develops is sterile. If such a gonad-forming area is permitted to develop as is done in the present grafting experiments, it forms a definite sex gland which is also sterile.

Can these results be accepted as proof of the extra-gonadal theory of origin of the germ cells in the chick? Certain evidence obtained from our graft studies indicates the necessity of exercising caution in making such an interpretation. In the first place such a conclusion is rather difficult to reconcile with the finding that a sterile gonad forms in those implants of the pellucid area which included the germ cell crescent. In such grafts the germ cells may be found scattered in the mesenchyme or missing altogether. Whether they are present in the graft makes no difference in the result. This is expected since the pellucid area in the graft develops no blood vascular system of its own, consequently the germ cells of the

crescent have little or no chance of getting to the developing gonad. The complete disappearance of the germ cells from the implant may be explained as the result of their entrance into the blood circulation of the host embryo or of phagocytic processes.

A second disturbing point in this connection is the discovery that a sterile gonad may form in a graft of the gonad-forming area at a stage when germ cells are already present in it (Willier, '33, p. 641). This has occurred in two cases out of seventy-five examined. Also in a few grafts a part of the same body of gonad tissue may be sterile. Since germ cells are found in the mesenchyme of these grafts, it is likely that they have migrated from the differentiating gonad-forming area, or a part thereof, leaving it sterile.

A third significant point is the occurrence of germ cells in a graft of a tiny median piece containing the anterior half of the node of a blastoderm of a head-process stage (Rawles, '36, p. 291). The germ cells found in such a graft are identical as regards structure to those found in lateral pieces which included some of the germinal crescent and are apparently similar to those occurring in grafts of the gonad-forming area itself.

The significance of this result is somewhat puzzling. It is conceivable in the first place (especially since these so-called germ cells appear in grafts from practically all but the most posterior regions of the pellucid area) that they represent a special type of cell characteristically developed in the grafted pieces. A second interpretation is that the node area, which seems to have considerable powers of reconstitution, forms germ cells *de novo*. It is suggested, in the third place, that the site of origin of germ cells is much more extensive than has been described by Swift and others. A preliminary study of the distribution of the primordial germ cells in blastoderms of pre-somite and early somite stages reveals that they are not confined to a narrow crescentic zone at the anterolateral boundary of the pellucid area but occur in small numbers medially and posteriorly from it (figs. 1 and 2). In view of

such a scattered distribution the results with the median piece can be fitted into the view that germ cells are extra-gonadal in origin.

A fourth consideration bearing on the question of germ cell origin is that conditions peculiar to the graft may inhibit the differentiation of germ cells similar to what appears to happen in the case of the gonad. The very small size of the gonad formed and low frequency of formation in the grafts of the present experiments, suggest that conditions are not ideal for its origination. This is apparently not due to the absence of the primordial germ cells since a similar low frequency was found to be characteristic of grafts of the gonad-forming area at a stage when germ cells are present in it (Willier, '33). There is evidently some condition lacking in grafted pieces from blastoderms prior to 72 hours incubation, that accounts for the failure of gonad differentiation. This is somewhat peculiar to gonad for under the same conditions mesonephros and suprarenal differentiate well and with a somewhat higher frequency. It seems reasonable to expect, therefore, that under such unfavorable circumstances germ cells might fail to differentiate.

A significant problem for discussion at this time is the nature of the so-called primordial germ cells in the chick. On this subject considerable controversy has arisen among investigators (for a recent review of the literature on this subject see Blocker, '33). The chief point at issue is whether these cells are germinal or non-germinal in nature. That these cells are germinal in nature from the time of their early segregation is the more commonly, but not too critically, accepted view. Swift ('15 and '16), Goldsmith ('28) and others claim not only to have traced their migration into the developing gonad rudiment but to have followed the cytological changes as they transform into definitive sex cells. An argument of more recent origin, based on the experiments of Benoit, Reagan and Dantschakoff, is that since injury or destruction of the germinal crescent results in the appearance of a gonad area free from germ cells, they must be the

forerunners of definitive sex cells. These descriptive and experimental studies seem to indicate that the primordial germ cells are the sole source of all definitive sex cells. That the experimentally produced sterile gonad rudiment may have the ability to form sex cells at some later stages is not ruled out. Indeed Fell ('23) and Gatenby ('24) have brought forward evidence that germ cells may arise from the peritoneum of the adult fowl. Others, such as Firket ('14 and '20) and de Winiwarter and Sainmont ('09) have arrived at an interpretation, not too convincing, for the existence of two sets of germ cells. The first or primary ones degenerate and are replaced by germ cells which arise from the epithelial components of the gonad. A related interpretation is that the definitive sex cells come from these two sources.

The strongest evidence for the interpretation that the primordial germ cells are the sole source of the definitive sex cells comes from an experimental analysis of the sexual nature of the right ovary of the domestic fowl. It is found that the primordial germ cells (assuming their extra-gonadal origin) persist in the right ovary until the third week after which they gradually disappear (Brode, '28). If the left ovary is excised before the time of their disappearance, the right ovary transforms into a testis containing male sex cells which may even reach the stage of mature spermatozoa; on the other hand, if removed after the time of their disappearance no sex cells whatever differentiate in the testis (Benoit, '23; Domm, '29).

The alternative view for examination is that primordial germ cells are non-germinal in nature. Berenberg-Gossler ('14), although able to confirm in all essentials Swift's account of their origin and migration to the genital ridges, regarded them not as germ cells at all but as entodermal wandering cells which transform late into mesodermal cells—and being like any other mesodermal cell may give rise to sex cells. In studying the development of the germ cells in the human testis Stieve ('27) reaches a similar conclusion.

It seems highly probable that the primordial germ cells are not germ cells in the strict sense but are relatively undifferentiated cells which remain in this condition until gonad differentiation sets in. This is beautifully brought out in ootestes produced in chick embryos by the injection of sex hormones (Willier et al., '37). In such gonads, irrespective of their zygotic sex constitution, the germ cells differentiate in the ovarian cortex into oogonia and in the testicular cords into spermatogonia. If by chance a germ cell comes to lie outside of either cortical or testicular tissue, i.e., in the mesenchyme, it remains undifferentiated. A similar lack of differentiation of germ cells is seen in grafts of the germinal crescent itself (Willier, '33). It is thus evident that the primitive germ cells not only have the capacity to differentiate in either the male or female direction but are dependent upon a specific sex-cord environment for their differentiation into specific sex cells. Such a labile state of the primordial germ cell is indicative of its generalized nature.

Granting the germinal nature of the primordial germ cells an interesting problem is presented as to the conditions of their origin at a particular time and place in the blastoderm. That they are conditioned in the direction of germ cells by correlative processes seems probable. Certainly such an interpretation is more in accord with the principle of correlative development, shown to hold in the determination of other parts of the embryo, than with the view that they represent a 'segregation of germ plasm' in the Weismannian sense. The nature of the processes involved in their origin remains for future elucidation.

Gonad differentiation independent of primordial germ cells. Although Reagan, Benoit and Dantschakoff have shown that the gonad-forming area of an embryo may be freed of germ cells by removing or destroying the germinal crescent, its power to differentiate into a gonad recognizable as to sex has hitherto not been ascertained. The failure of the embryos to live long following the destruction of the germinal crescent largely explains the lack of data on this point. On this

problem Dantschakoff ('31, '32) has argued that since no gonad rudiment forms when the entire crescent is cauterized and one with a reduced number of germ cells forms when the germinal crescent is only two-thirds or three-fourths destroyed, the entodermal wandering cells (germ cells) act as an 'organizer' of the gonad. In other words the germinal epithelium alone cannot 'organize' a gonad. In most of the cases of complete cauterization of the crescent the embryos are, it seems probable, not old enough at the time of their examination—in most cases less than 80 hours old—to have formed a distinct gonad rudiment. The injuries brought about by the drastic treatment necessarily given such embryos has certainly retarded their development as compared with the normal. According to our studies the germinal epithelium makes its first appearance at approximately the 35-somite stage as a definite thickening of the coelomic epithelium. This stage may be reached in embryos incubated from 60 hours (Willier, '33) to 84 hours (Swift, '15) depending upon the temperature of incubation, season of the year, and other variables.

The abnormal conditions, especially the changes in vascular circulation, may inhibit the development of the gonad rudiment in such embryos. That the origination of the gonad rudiment is easily inhibited is indicated by certain studies on its frequency of formation in chorio-allantoic grafts. In grafts containing the portion of the embryo normally forming the adrenal-gonad-mesonephros complex, i.e., the level between the fifteenth somite and the node of embryos of 15- to 17-somite stages, mesonephros differentiates in the majority of cases, adrenal in half of the cases and gonad somewhat rarely. It is evident therefore that these results of Dantschakoff cannot be accepted as proving that the gonad rudiment is unable to develop in the absence of primordial germ cells.

On the contrary the gonad rudiment not only has the power to arise but it may differentiate into a sterile gonad independently of primordial germ cells. This has been shown by testing in chorio-allantoic grafts the power of the whole

pellucid area or a certain small portion of it at selected stages in its development to differentiate gonad. The stages chosen were either early somite or head process, all prior to any possible migration of the primordial germ cells from their seat of origin and prior to the formation of the gonad-forming areas. Whether the germinal crescent is removed from the implants of the whole pellucid area seems to make no difference in the result, since if it is included there is no mechanism for transporting the germ cells to the developing gonad areas. The miniature sterile body which has formed in a total of ten cases, is without doubt a gonad as its structural organization and topographical position, i.e., in juxtaposition to mesonephros and adrenal, conclusively demonstrate. The small size of the gonad is not to be correlated with the absence of germ cells, since it has been shown that a transplanted gonad-forming area in which they are present likewise forms a small gonad. As previously pointed out (Willier, '33, p. 636) differentiation without growth in chorio-allantoic grafts is the rule not only for gonad but for other organ-forming systems.

Comparative studies on other animals also show that a gonad may arise independently of the primordial germ cells. In the amphibians both Kuschakewitsch ('10) and Humphrey ('27, '28) have reported the differentiation of a sterile gonad or its rudiment under certain experimental conditions in which the germ cells either failed to separate from the entoderm or their exact history is unknown. A more convincing demonstration is furnished by the experiments of Geigy ('31) on the eggs of *Drosophila melanogaster*. By exposure to ultra-violet radiation the primitive sex cells situated at the germinal pole of the egg may be destroyed. If all of these cells are destroyed the eggs develop normally except that both lobes of the gonad of both sexes are sterile. If the sex cells of one side only are destroyed the lobe of the gonad of the corresponding side only is sterile. Although retarded to some extent in development such sterile ovaries and testes are normal in form and histology. Similarly in the frog Bour-noure ('37) finds that the somatic elements may form a gonad

'pratiquement privées de tissu fertile' following ultraviolet irradiation of the inferior pole of the unsegmented egg.

A question related to the one just discussed is the dependence of the gonad rudiment upon the mesonephros for its origin and differentiation. Dantschakoff ('32) in her theory that all of the component parts (coelomic epithelium, entodermal wandering cells and mesonephros) are essential for gonad development would support this view. So far as our evidence goes it is clear that a gonad rudiment may differentiate somewhat independently of the mesonephros, since in no case are urinogenital connections developed and in a number of instances the gonad is somewhat distantly removed from the mesonephros. That a gonad rudiment may arise entirely independently of the mesonephros is difficult to conceive. Indeed Dantschakoff ('32) has found that the gonad anlage separated from the mesonephros from embryos of $3\frac{1}{2}$ days, fails in chorio-allantoic grafts to form a gonad with sex cords. Whether the failure to form a gonad is due to a lack of correlative influences or to injury effects to the gonad-forming areas during their isolation cannot of course be decided.

A final problem of considerable interest for discussion is whether the germ cells have a stimulative action upon non-germinal cells and tissues such as the coelomic epithelium or mesenchyme. This has been discussed somewhat fully in a previous paper (Willier, '33, p. 639 ff.) and the conclusion reached that the evidence for any such activation is inadequate.

The singular differentiation of gonads structurally male. One of the most interesting results for analysis is the invariable development of a testis-like gonad, ovarian cortex having failed to differentiate in each of the ten cases examined. This is the case regardless of the method used for obtaining them. Two possible interpretations suggest themselves. First, these testis-like gonads are to be regarded as having a male chromosome composition, a differential survival of the sexes in favor of the males having occurred. Second, on the basis of the expected sex ratio, approximately half of these

should possess the female chromosome composition. On statistical grounds it seems somewhat improbable, although theoretically possible, that all of these gonads should have a male-determining constitution. Repeated attempts have been made to ascertain the nature of the chromosome composition of the gonads and although mitotic figures are moderately abundant, none is sufficiently ideal to enable one to distinguish with certainty the sex chromosomes. Should it be established that some of the gonads have a female chromosome composition, a very significant problem in gonad differentiation presents itself for analysis, namely: the inability to form ovarian cortex in the absence of the primordial germ cells. It may well be that ovarian cortex which in the normal contains by far a greater number of germ cells than the medullary cords, is unable to form in their absence. Lastly, it should be pointed out that upon the failure of ovarian cortex to differentiate, the medullary cords would be expected to assume the male structural form of the primary sex cord since such a transformation is known to occur in the right ovary following the removal of the left ovary in female chicks after hatching. In this manner the invariable development of a male structural composition of the sterile gonads may be explained.

SUMMARY

1. Isolated portions of chick blastoderms at certain critical stages in the developmental history of the primordial germ cells were tested in the chorio-allantoic graft for their power to form a gonad.

The stages selected include blastoderms in which the primordial germ cells are 1) still at or near the seat of their origin, the germinal crescent (head process to 10 somites), 2) in the blood vessels (10 somites to 25 somites) and 3) in the gonad-forming areas.

A variety of isolated portions were analyzed: 1) The entire pellucid area including a small part or all of the germinal crescent, 2) the entire pellucid area except for the head region and entire germinal crescent, 3) the pellucid area between fifteenth somite level and node (12 to 25 somite donors),

4) a tiny piece containing the primitive knot of the head-process blastoderm and 5) gonad-forming areas of from 29- to 41-somite donors. The last mentioned are introduced for a comparative study (Willier, '33).

2. Regardless of the nature of the isolated piece from the pellucid area the gonad which differentiates is invariably sterile and consists of male-like sex cords, ovarian cortex never having formed. This has occurred in ten cases out of a total of eighty-six grafts, showing a low frequency of origin. Typically the gonad lies adjacent to mesonephros and supra-renal gland. The host may be either male or female. Whether the germinal crescent is included in the implant or whether the germ cells persist seems to make no difference in the result obtained, since in all cases the gonad is sterile. This is accounted for in part by the lack of development in the graft of a mechanism for transporting the primordial germ cells to the developing gonad areas.

3. The formation of a sterile sex gland from the gonad area in the absence of primordial germ cells supports Swift's theory of their extra-gonadal origin. This is not regarded, however, as a crucial experiment, since gonad development is apparently easily and singularly inhibited by conditions peculiar to the graft, which likewise may inhibit the differentiation of germ cells.

The nature of the primordial germ cell is discussed and the suggestion made that it is a generalized cell which is dependent upon correlative processes for its origin and for its subsequent differentiation into a specific sex cell.

4. The result indicates that a gonad rudiment not only has the power to arise but may differentiate independently of the primordial germ cells. The relationship of gonad origin and differentiation to the mesonephros is discussed and the conclusion reached that although dependent at first, subsequent differentiation is probably independent of this body.

5. The invariable differentiation of gonads structurally male is an unexpected result. Granting the probability that some of them have a female-determining constitution, a significant problem in gonad origin presents itself for analysis,

viz., the inability to form ovarian cortex in the absence of germ cells, and the assumption by the primary sex cords of a male rather than a female structure.

LITERATURE CITED

- BENOIT, J. 1923 Sur la structure histologique d'un organe de nature testiculaire développée spontanément une poule ovariectomisée. C. R. Acad. Sc., T. 177, pp. 1243-1246.
- 1930 Destruction des gonocytes primaires dans le blastoderme du poulet par les rayons ultraviolets, aux premiers stades du développement embryonnaire. Proc. 2nd. Int. Congress for Sex Research, pp. 162-170.
- BERENBERG-GOSSLER, H. 1914 Über Herkunft und Wesen der sogenannten primären Urgeschlechtszellen der Amnioten. Anat. Anz., Bd. 47, S. 241-264.
- BLOCKER, H. W. 1933 Embryonic history of the germ cells in *Passer domesticus* (L). Acta Zoologica, vol. 14, pp. 111-162.
- BOURNOURE, M. L. 1937 La constitution des glandes génitales chez la Grenouille rousse après destruction étendue de la lignée germinale par l'action des rayons ultraviolets sur l'oeuf. C. R. Acad. Sc., T. 204, pp. 1957-1959.
- BRODE, M. D. 1928 The significance of the asymmetry of the ovaries of the fowl. J. Morph., vol. 46, pp. 1-57.
- ✓ DANTSCHAKOFF, W. 1908 Untersuchungen über die Entwicklung des Blutes und Bindegewebes bei den Vögeln. I. Die erste Entstehung der Blutzellen beim Hühnerembryo und der Dottersack als Blutbildendes Organ. Anat. Hefte, Bd. 37, S. 471-589.
- 1932 Keimzelle und Gonade. II b. Ganzheit des Gewebekomplexes als Faktor in der Entwicklung der Gonade. Zeit. f. Zellforsch. u. mikr. Anat., Bd. 15, S. 581-644.
- ✓ DANTSCHAKOFF, W., W. DANTSCHAKOFF, JR. AND L. BERESKINA 1931 Keimzelle und Gonade. I A. Identität der Wanderzellen und der entodermalen Wanderzellen. Experimentelle Beweise. Zeit. f. Zellforsch. u. mikr. Anat., Bd. 14, S. 323-375.
- DOMM, L. V. 1929 Spermatogenesis following early ovariectomy in the brown leghorn fowl. Arch. f. Entwick-mech. d. Organ., Bd. 119, S. 171-187.
- FIRKET, JEAN 1914 Recherches sur l'organogenèse des glandes sexuelles chez les oiseaux. Arch. de Biologie, T. 29, pp. 201-351.
- 1920 On the origin of germ cells in higher vertebrates. Anat. Rec., vol. 18, pp. 309-316.
- FELL, H. B. 1923 Histological studies on the gonads of the fowl. I. Histological basis of sex-reversal. Brit. J. Exp. Biol., vol. 1, pp. 97-130.
- GATENBY, J. BRONTÉ 1924 The transition of peritoneal cells into germ cells in *Gallus bankiva*. Quart. J. Micr. Sci., vol. 68, pp. 1-16.
- GEIGY, R. 1931 Action de l'ultra-violet sur le pôle germinale dans l'oeuf de *Drosophila melanogaster* (castration et mutabilité). Revue Suisse de Zoologie, T. 38, pp. 189-288.

- GOLDSMITH, J. B. 1928 The history of the germ cells in the domestic fowl. *J. Morph.*, vol. 46, pp. 275-315.
- HUMPHREY, R. R. 1927 Extirpation of the primordial germ cells of *Amblystoma*: its effect upon the development of the gonad. *J. Exp. Zool.*, vol. 49, pp. 363-399.
- 1928 The developmental potencies of the intermediate mesoderm of *Amblystoma* when transplanted into ventrolateral sites in other embryos: The primordial germ cells of such grafts and their role in the development of a gonad. *Anat. Rec.*, vol. 40, pp. 67-101.
- HUNT, T. E. 1931 An experimental study of the independent differentiation of the isolated Hensen's node and its relation to the formation of axial and non-axial parts in the chick embryo. *J. Exp. Zool.*, vol. 59, pp. 395-428.
- KUSCHAKEWITSCH, S. 1910 Die Entwicklungsgeschichte der Keimdrüsen von *Rana esculenta*. *Festschrift R. Hertwig, Jena*, Bd. 2, S. 61-224.
- RAWLES, MARY E. 1936 A study in the localization of organ-forming areas in the chick blastoderm of the head-process stage. *J. Exp. Zool.*, vol. 72, pp. 271-316.
- REAGAN, F. P. 1916 Some results and possibilities of early embryonic castration. *Anat. Rec.*, vol. 11, pp. 489-491.
- STIEVE, H. 1927 Die Entwicklung der Keimzellen und der Zwischenzellen in der Hodenanlage des Menschen. Ein Beitrag zur Keimbahnfrage. *Zeit. mikr. Anat. Forsch.*, Bd. 10, S. 224-285.
- SWIFT, C. H. 1914 Origin and early history of the primordial germ cells of the chick. *Am. J. Anat.*, vol. 15, pp. 483-516.
- 1915 Origin of the definitive sex cells in the female chick and their relation to the primordial germ-cells. *Am. J. Anat.*, vol. 18, pp. 441-470.
- 1916 Origin of the sex cords and definitive spermatogonia in the male chick. *Am. J. Anat.*, vol. 20, pp. 375-410.
- WILLIER, B. H. 1926 The development of implanted chick embryos following the removal of the 'primordial germ cells.' *Anat. Rec.*, vol. 34, p. 158.
- 1932 The embryological foundations of sex in vertebrates. Chap. IV, Sex and Internal Secretions, edited by E. Allen. Williams and Wilkins Co.
- 1933 a Potencies of the gonad-forming area in the chick as tested in chorio-allantoic grafts. *Arch. Entw.-mech.*, Bd. 130, S. 616-648.
- 1933 b On the origin and differentiation of the sexual gland. *Am. Nat.*, vol. 67, pp. 1-8.
- WILLIER, B. H., T. F. GALLAGHER AND F. C. KOCH 1937 The modification of sex development in the chick embryo by male and female sex hormones. *Physiol. Zool.*, vol. 10, pp. 101-122.
- WILLIER, B. H., AND MARY E. RAWLES 1931 The relation of Hensen's node to the differentiating capacity of whole chick blastoderms as studied in chorio-allantoic grafts. *J. Exp. Zool.*, vol. 59, pp. 429-465.
- 1936 The distribution of the so-called primordial germ cells in early chick blastoderms and their relationship to sterile gonad formation in chorio-allantoic grafts. *Anat. Rec.*, vol. 64 (Suppl.), p. 77.
- DE WINIWARTER, H. AND G. SAINMONT 1909 Nouvelles recherches sur l'ovogenèse et l'organogenèse de l'ovaire des mammifères (chat). *Arch. de Biol.*, T. 24, pp. 1-143.

STUDIES ON THE REPRODUCTIVE SYSTEM OF THE ALLIGATOR

I. THE EFFECTS OF PROLONGED INJECTIONS OF PITUITARY WHOLE GLAND EXTRACT IN THE IMMATURE ALLIGATOR

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ONE TEXT FIGURE AND TWO PLATES (SIX FIGURES)

INTRODUCTION

Numerous experiments, particularly with the higher vertebrates, have established the fact that, in the forms investigated, the gonads depend for their development to the adult condition and for their proper maintenance and function on one or more of the secretions of the hypophysis. The pituitary-gonad relationship in reptiles has received relatively little attention. With the exception of a preliminary paper by the author (Forbes, '34), no information on this subject as it relates to the alligator has been published.

Houssay reported in 1930 that five homeoplastic hypophyseal implants into a single snake, *Xenodon merremi*, brought about ovulation in 6 days. Injections of a pituitary anterior lobe extract into several adult male *Lacerta* during the non-breeding period provoked the sexual segments of the renal collecting tubules (accessory sex structures in certain reptiles) to the active secretory condition (Herlant, '33). Injections of human pregnant urine (known to contain a gonadotropic substance) into male blindworms of the genus *Anguis* produced a similar activity in both the sexual segment and the epididymis and an increase in interstitial tissue. Administration of the same substance to *Lacerta* males was followed by hypertrophy of the interstitial tissue.

Schaefer ('33) has reported that hypophysectomy of male garter snakes (*Thamnophis sirtalis* and *T. radix*) resulted in testicular atrophy. Partial restoration of the gonads to the normal condition was accomplished by repeated subcutaneous hypophyseal implantations. Cunningham and Smart ('34) induced ovulation in *Lacerta viridis* and *Anguis fragilis* by the injection of acetic acid anterior lobe extract. The administration of hypophysis products to male palm lizards of the genus *Uromastix* brought about spermatogenesis during the normal seasonal quiescent period (Kehl, '35).

Evans ('35 a, b) injected males and females of *Anolis carolinensis* with either Antuitrin S or whole gland sheep pituitary extract. In both sexes the gonads were hypertrophied and the accessory sex structures were markedly stimulated. Injections of Antuitrin S into the lizard *Eumeces laticeps* during the period of sexual quiescence brought about a marked increase in the weight of testes and accessories in the male and a restoration of the oviducts in the female to approximately the condition obtaining during the breeding season (Turner, '35). Clausen ('35) found that hypophysectomy of gestating viviparous snakes, unless performed shortly before parturition, prevented the birth of normal living young; the injection of hypophysis extracts during the later stages of gestation caused immediate birth of the young. The administration during the period of sexual quiescence of hog anterior pituitary extract to the horned lizard, *Phrynosoma cornutum*, combined with continuous exposure to light and heat and an over-supply of food, resulted in a marked stimulation of the gonads and gonoducts (Mellish, '36).

It is apparent from these experiments that the gonads of adult reptiles, like those of other vertebrates, not only depend for their normal maintenance and function on the presence of a gonadotropic hormone or hormones but may be definitely stimulated when such hormones are administered experimentally. Thus far no attempt to stimulate the gonads of a sexually immature reptile has been reported except by the author (Forbes, '34). The latter's experiments were undertaken particularly to investigate the possibility of inducing in

the immature alligator a precocious development of the reproductive tract similar to that reported in other immature vertebrates following administration of hypophyseal extracts.

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MATERIAL AND METHODS

Two experiments were carried out, one on a younger and one on an older group of *Alligator mississippiensis*. The sex of the immature animal cannot be learned by external examination, and hence the ratio of males to females in each group was purely fortuitous.

The younger group of animals, approximately 4 months old and of an average total length of 24 cm. at the beginning of the injection period, consisted of ten control and ten experimental alligators. Three times a week, on alternate days, 0.5 cc. of alkaline extract of whole sheep hypophysis was injected intraperitoneally into each animal. Two experimental animals were sacrificed, and one experimental and one control animal died, during the course of the experiment. After 6 weeks of injections, each animal having received a total of 9 cc. of extract, followed by 2 weeks without injections, all animals were sacrificed and the reproductive tracts preserved in Bouin's fluid. The tissues were serially sectioned at 10 μ and stained with haematoxylin and eosin.

A similar experiment was carried out with a second group of alligators which were approximately 18 months old and of an average total length of 48 cm. when the injections were begun. There were ten control and ten experimental animals; each of the latter received 1.0 cc. of the whole gland alkaline pituitary extract six times a week until a total of 39 cc. of extract had been administered. One experimental animal died during the course of the injections.

Autopsy of the surviving animals showed that in the younger group there were four male and five female controls and one male and six female experimental animals, while in the older group there were nine male and one female controls and seven male and two female experimental animals.

The degree of immaturity of the two groups of animals is indicated by the belief (Reese, '15; McIlhenny, '35) that the sexually adult condition is not attained by the female alligator until an age of at least 5 or 6 years. Precise information on this point is not available.

The pituitary whole gland alkaline extract used in the experiments was supplied through the courtesy of Dr. E. P. Bugbee of Parke, Davis & Company.

RESULTS

A. Macroscopic observations

Macroscopic examination at autopsy of the younger group of alligators immediately revealed a marked hypertrophy of the gonads of the experimental animals. The testes of the single experimental male in this group were double the length, width and thickness of those of the control males. The six females showed definitely hypertrophied ovaries, although the degree of difference in size between control and experimental ovaries was not so great as in the case of the males. The ovarian development of the experimental females was particularly marked by the manner in which the gonads were enlarged and extended posteriorly. The oviducts were hypertrophied, and one or both oviducts in each animal had become definitely convoluted.

Autopsy of the older group likewise showed an enormous hypertrophy of the testes in the males (fig. 2) and of the ovaries and oviducts in the females (fig. 3). The vasa deferentia of the experimental males did not differ macroscopically from those of the controls. The degree of hypertrophy of the gonads of the older animals was considerably greater than in the first group, due both to their more advanced age and to

the much larger amount of hormone with which they were injected.

An additional effect produced in the injected animals of the older series was a loss, partial or complete, of the yellow pigmentation forming the bright transverse dorsal stripes observed on normal young animals. In many cases the dorsal integument was a uniform black. The stripes appeared dimly on the sides of the animals. It is presumed that this effect was due to the presence in the pituitary extract of the pigment-regulating substance of the intermediate lobe of the hypophysis.

Because of fundamental similarity to the older group, no figures showing the younger animals have been included.

B. Microscopic observations on the gonads

1. *The younger series.* Microscopic study of the serial sections of gonads and gonoducts and nearby tissues made the relationships of various organs more apparent. A mesonephros with large glomeruli is still present and appears functional, although the metanephros is of course much more prominent. The mesonephros is directly dorsal to the gonad, which covers the ventrolateral mesonephric surface, while the adrenal body lies medial to the mesonephros. All the organs are closely apposed. The anterior end of the adrenal gland extends a short distance cephalad to the anterior end of the gonad, and the posterior pole of the gonad extends some distance caudad beyond the end of the adrenal.

a. *The control males.* The testis of a control male of this age consists of an external layer of germinal epithelium; the latter lies external to a tunica albuginea, which in turn invests the zone of medullary sex cords. The medulla forms the greater part of the volume of the testis.

The germinal epithelium consists for the most part of a single layer of cells. The nuclei lie close together, are larger than those of the underlying tunica albuginea, and stain deeply with haematoxylin. Occasionally the cells of the germinal epithelium are found to be stratified in sharply delimited areas

so as to give the appearance of nests of cells lying on, and in some cases embedded in, the tunica albuginea. It was found in somewhat older animals that the local nests of cells are more frequent, indicating that the germinal epithelium is by no means inactive in males of the ages studied. The nests contain from four to eight germ cells, which are characterized by their large nuclei containing chromatin granules and by their clear cytoplasm. Germ cells, individually and in groups of three or four, are also scattered through the medullary cords. The tunica albuginea is thin, except at the lateral line of separation of testis and mesonephros, and does not as yet show a marked development. It presents the usual appearance of a lamina of dense connective tissue. The cells are comparatively small, and the nuclei are long and spindle-shaped. Beneath the tunica is the zone of medullary sex cords, which lie at right angles to the tunica and are the forerunners of the seminiferous tubules. A few of the latter are already present, but in a primitive state, the lumina being small and the tubule contents without definite organization. However, the whole sex cord area shows activity and growth, as evidenced by crowding of the cells, numerous mitotic figures, and some anastomosis of the cords.

At its posterior end, the gonad is present only in the primitive undifferentiated form, i.e., the germinal epithelium, lying closely adherent to the surface of the mesonephros, has not yet given rise to the medullary or primary sex cords characteristic of the anterior end of the testis.

b. The experimental male. Histological sections from the single experimental male of the younger group present quite a different picture. The most obvious effect of the injections is a considerable hypertrophy of the testes. The mass of medullary sex cords is greatly increased, and the individual cords are thicker than those of the controls. A narrow lumen is present in many of the cords. The number of germ cells is not increased out of proportion to the gonad hypertrophy. The nests of germ cells at the periphery and within the gonad have disappeared, the cells now being scattered individually

or in pairs. Unpublished evidence indicates that the scattered nests of germ cells appearing on and in the tunica albuginea of the control animals represent patches of potential cortex retained from the bisexual condition during prehatching stages. The tunica albuginea is very thin in many areas, and in fact cannot everywhere be recognized as a continuous layer.

The foregoing observations indicate that the various changes described are those proper to normal development, but that these changes have been markedly accelerated by the experimental treatment. No conclusions should be drawn solely from the results in the single experimental male; however, these results agree with and are supplemented by those obtained from the older group.

The peritoneum visible in sections from the experimental animals is seen to have undergone marked changes. The serosa of the control animals exists as a typical single layer of mesothelial cells. In the experimental animal, however, particularly over the gonads, the peritoneal layer has become thickened by hyperplasia until it is several cell layers deep. In many regions this layer is partially detached, due to sloughing of the tissue. The thickened peritoneal layers are invaded by lymphocytes. It is believed that these pathological changes in the serosa are due to a chronic irritation of the latter by unabsorbed substances in the extract introduced into the abdominal cavity, and indeed a white coagulum was found at autopsy partly covering the abdominal viscera.

c. The control females. An examination of sections of control ovaries from the younger age group shows a germinal epithelium of the tall simple columnar type. Interspersed between the columnar cells at irregular intervals are large germ cells, characterized by their size, large nuclei and clear cytoplasm. These oogonia may occur singly, in pairs or in clusters of three or four or more. In the latter case, the nest of cells projects slightly into the substance of the ovary proper. Germ cells are not to be seen except at the extreme periphery of the ovary, and at this period in the development of the female gonad, the secondary sex cords are only slightly

developed. The latter are seen as short conical projections toward the hilum of the ovary from the smooth inner surface of the cortical (epithelial) layer. Nevertheless, the tunica albuginea is less definitely developed, and the secondary sex cords have begun to perforate it. The nests of germ cells mentioned above are very similar to the smaller aggregations of spermatogonia described at the surface of the testes of animals of this age.

Within the control ovary, remnants of the medullary cords (primary sex cords) may be seen. The latter, together with some connective tissue, comprise the ovarian medulla, persistent at this stage. The medulla is not solid; the cords contain numerous lacunae. A definite line of demarcation, as indicated by differences in tissue structure, may be seen between the ovary and the immediately subjacent mesonephric stroma.

d. The experimental females. As in the case of the experimental male, the injected females of the younger group exhibit a marked hypertrophy of the gonads. The increase in size, while not so great as in the male, is nevertheless very evident, involving particularly as it does a lengthening of the organs posteriorly. A serosal hyperplasia of the type observed in the experimental male is again seen to occur in the injected females.

The germinal epithelium and medulla are both hypertrophied, but it is evident that much of the cortex has been eroded by local reaction to the irritating residues previously referred to. The irregular surface of the germinal epithelium may also be due to rapid cell proliferation and unequal growth; such activity would perhaps account for the jagged clefts with which the germinal epithelium is marked. Although these irregularities may be accentuated by technical procedures, yet, since they do not occur in adjacent tissues of the same sections, it is thought that the condition is largely the result of rapid, uneven growth. The secondary sex cords are not developed to a greater extent than in the controls, and again are seen as short conical projections into the medulla

from the internal surface of the cortex, which at this stage is represented only by the thickened layer of stratified germinal epithelium. The boundary between cortex and medulla is quite definite, even though the tunica albuginea cannot be recognized, since in the layer of cortical cells in contact with the periphery of the medulla the nuclei are withdrawn into the outer or distal ends of the columnar cells; thus, as a result, the clear cytoplasm of the proximal ends of the cells forms a light band which marks the internal boundary of the cortex. The number of oogonia is not greater than in the control ovaries. These cells may be scattered through the cortex or may occur in clumps in the primitive secondary sex cords. Germ cells are not to be seen in the medulla. The medullary cords, as in the controls, are irregular and anastomotic.

2. *The older series.* a. The control males. The gonads of the control males of the older group show a more advanced stage of development. The tunica albuginea is now seen to be a dense layer of laminated connective tissue which covers the surface of the testis (fig. 4). External to and investing the tunica is a typical serosa. The germinal epithelium as such has disappeared except in a few isolated areas.

These exceptional regions are important and require explanation. At intervals the tunica albuginea is seen to be perforated by a column of cells which continues through the tunica and into the medulla (fig. 4). External to the tunica, the column spreads out into a flattened disc which extends for a short distance over the surface of the gonad. Thus the whole structure has a fungiform appearance. It will be recalled that in the control males of the younger series, areas of germinal epithelium were noted on the surface of the tunica albuginea. It is believed that the columns of cells extending from similar areas into the medulla in the older males represent isolated cortical proliferations homologous to the secondary sex cords of normal female animals. This belief is strengthened by the occurrence of germ cells in the proliferated cords.

Most of the medullary sex cords have now become seminiferous tubules. The lumina of the latter are filled with germ cells and small amounts of cellular debris (fig. 4). The tubules are remarkably tortuous and occasionally anastomose. Little if any interstitial tissue is present.

b. The experimental males. The testes of the male animals of the experimental group show enormous hypertrophy (fig. 5). The gonads have increased in length, width and thickness. The diameters of the seminiferous tubules are several times those of the non-stimulated tubules. The lumina of the tubules are crowded tightly with free spermatogonia, and additional germ cells are interspersed among the Sertoli cells. The zoning of germ cells, characteristic of typical tubules, according to the various stages of spermatogenesis is not apparent. The densely staining chromatin granules of the germ cells are conspicuous.

The tunica albuginea is two to three times as thick as in the control males and is continuous with the dense septula testis, which separate the lobules of the testis. The septula are irregular and occasionally discontinuous, due to the fact that the seminiferous tubules are tortuous and anastomotic (fig. 5).

The serosa overlying the gonad consists of typical flattened epithelial cells and shows no signs of irritation by residues from the extract injected such as occurred in the younger group. The germinal epithelium is no longer present except in the fungiform patches referred to, lying external to and in the tunica albuginea and continuing as cords into the medulla. The number of epithelial areas is approximately the same as in the controls. These structures were described in connection with the control male animals. The persistent germinal epithelium has responded slightly to hormonal stimulation by the proliferation of cells and the development of the pedicle-like stalk into a small tubule.

c. The control female. The ovaries of the single control female of the older group are invested on their free surface by a typical serosa (fig. 6). Undifferentiated germinal epithelium is not present as a definite layer, but a few of the secondary sex cords can be recognized. The chief feature of the

ovary is the growing follicles, which contain large ovocytes. The cytoplasm of the latter stains lightly with eosin. A peculiar feature of the cytoplasm is that a cap-like disc partly surrounding the nucleus stains deeply with eosin and appears as a sharply defined red area in marked contrast to the light pink of the adjacent cytoplasm (fig. 6). Such an area of granular aggregation would seem to be similar to that described by Willier for the bird ('32, p. 153). The nuclei of the ovocytes are large and contain a dense network of threads and granules of chromatin. The follicular wall consists of a thin layer of flattened and stratified cells. The follicles themselves occupy most of the ovary, but between them can be seen stromal cells and oogonia, singly and in groups. The nuclei of these small germ cells frequently show mitotic figures.

Most of the original medullary region of the ovary is now represented by a loose network of connective tissue strands which extend radially from the mesonephros to the cortex and which represent the original medullary cords (fig. 6). However, beginning just anterior to the posterior tip of the cortex and extending a short distance caudal to the latter, the medulla persists as a solid mass of partially disorganized primary sex cords. The cords are composed chiefly of sustentacular cells, but germ cells are present in a primitive form. The cords are incompletely separated by strands of connective tissue. The free medullary tissue posterior to the cortex is covered by a simple layer of peritoneum, beneath which lies a thin tunica albuginea. No germinal epithelium is present in this region as a definite stratum. Where cortex and persistent medulla overlap, they are in direct contact. The unusual medullary area here described will hereafter be referred to as a medullary rest.

These observations are based on a single female in this group but are confirmed by similar findings in normal females of the same age which served as controls in another experiment.

d. The experimental females. The ovaries of the two injected females are by no means similar. The ovaries of the

first animal differ chiefly from those of the single control animal in that the cortex of the former has responded to stimulation by a marked increase in its volume (fig. 7). The number of large follicles appears no greater than in the control animal. The secondary sex cords have increased in size. The medulla exists peripherally as a thin stratum of solid tissue directly underlying the cortex, but continues centrally (toward the mesonephros) only as thin, widely separated strands of tissue, representing transformed medullary cords, which converge in the region of the wolffian body. Due to the fact that the cortex has grown much more than the medullary strands, the former, bound to the mesonephros by these strands, has become strongly arched, and the strands are drawn taut (fig. 7). Wide gaps exist between them. The medullary rest shows little response to stimulation.

The cortices of the second experimental female are unusual in that follicles with their large, prominent ova are almost entirely absent. On the other hand, the small undeveloped germ cells are more numerous than in the single control female animal, although these differences may be within the limits of individual variation. The ovarian cortex of this experimental animal is broader, but much thinner, than that of the control female, so that there has been no actual increase in cortical volume in the injected animal, although the gross appearance of the ovaries of the latter indicates an increase in their transverse dimension. The cortex is again arched, and the medullary strands are drawn taut. The medullary rest shows no hypertrophy.

In spite of the fact that this animal appeared externally to be uniform with the others used in the experiment, it is nevertheless believed that its sexual differentiation had not progressed to a stage much beyond that of the control animals of the younger series, even though, at the beginning of the experiment, the gonads were presumably of about the same size as those of females in the older group. There is no indication that follicles were present at the beginning of the experiment and disappeared due to the effect of the injections. It is

possible that diet and long captivity were factors in the abnormal condition of this animal. In other respects, the two experimental animals were not essentially different. There is little indication in either of any serosal irritation by extract residues.

It is obviously unwise to attempt to draw conclusions solely on the basis of results observed in only two experimental females. However, the results obtained in the experimental females of the younger group in general support the findings discussed above.

C. Quantitative estimates of growth of gonad components

In order to obtain precise information as to the relative hypertrophy of cortex and medulla in the gonads of the experimental animals when compared with controls, regularly spaced camera lucida outline tracings from the sections were prepared and weighed. The tracings represent accurately the relative growth of medullary and cortical zones except for the very thin single layer of germinal epithelium in the males and control females of the younger group and the small, infrequent cortical rests observed in the males of both groups. These latter tissues were considered to be of too slight volume to be statistically significant, and in addition were in quite different stages of development in the various animals. Tracings were made either of all or of a significant number of individuals of each sex group.

The values presented below are averages derived from a large number of individual measurements and are directly proportional to the volumes of the respective gonad components. Since much of the ovarian cortex of the experimental females of the younger series was destroyed by an erosive action of the injection material, as already described, the response of this tissue to stimulation is not fully indicated by the weight of its tracings. In addition to the cortex of the ovaries of the older series, two other tissues were measured: 'medullary strands,' i.e., the typical medulla, consisting of thin, widely separated strands of connective tissue, and

'medullary rest,' i.e., the testis-like area of solid medullary tissue at the posterior end of the ovary.

If the values expressed in the graph are now directly compared, the resulting ratios will give numerical expression to the relative degrees of hypertrophy of the various gonad components.

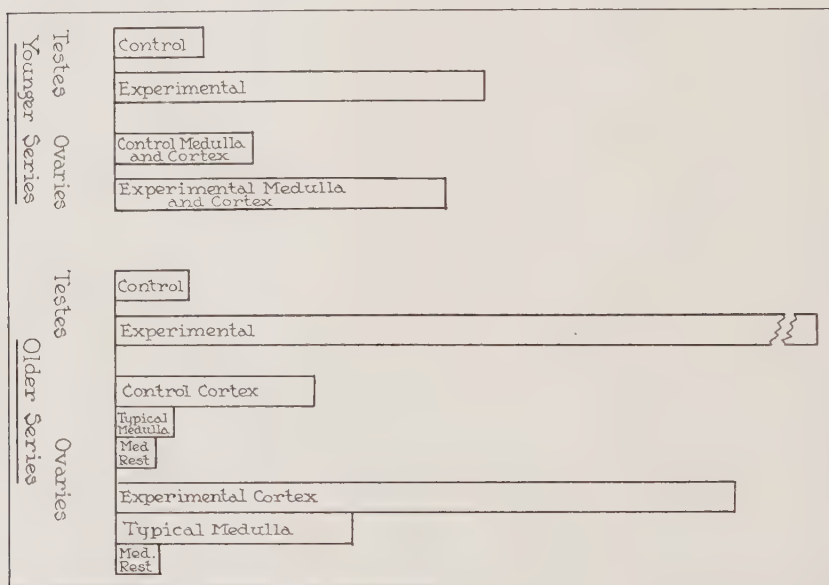


Fig. 1 Graphs to show relative average volumes of gonads and gonad components in the two series. The two graphs are not drawn to the same scale. The average volumes represented by the columns were obtained from individual weights of camera lucida tracings on paper of serial sections. The average volume of an experimental testis of the older series is actually about twenty-seven times as great as that of the control testis represented. The data are discussed in the text.

These data make evident a number of interesting facts. It is of course obvious that the experimental treatment has produced a definite, and in some cases an extreme, hypertrophy, particularly of the medullary tissue of the testis. It should be recalled that each animal of the younger series received a total dose of 9 cc. of extract, while each individual of the older group received 39 cc. of extract. The greater response of the latter group is probably due not only to greater dosage but

also to the more advanced development of the older gonads, which renders them more susceptible to stimulation.

It may also be seen that the testis has responded to a greater degree than the ovary, both relatively and absolutely, since, while the control testis is smaller than the control ovary, yet the experimental testis is larger than the experimental ovary. It will be noted that the medullary rest shows no significant response to stimulation.

Due to the destruction of the cortex of the experimental ovaries of the younger series and to the low number of experimental females in the older series, it is difficult to draw

TABLE 1

Series H

Control testis: experimental testis	1: 4.10
Control ovary: experimental ovary (total)	1: 2.39
Control testis: control ovary	1: 1.53
Experimental testis: experimental ovary (total)	1: 0.89

Series P

Control testis: experimental testis	1: 27.47
Control ovary: experimental ovary	
Medullary strands: medullary strands	1: 5.96
Medullary rest: medullary rest	1: 1.09
Cortex: cortex	1: 1.96
Total: total	1: 2.65
Control testis: control ovary (total)	1: 4.14
Experimental testis: experimental ovary (total)	1: 0.39

definite conclusions as to the relative response of cortex and medulla in the experimental ovaries. Furthermore, at least part of the increase in medullary volume is due to the mechanical stretching of the medulla by the rapid growth and resultant bulging of the cortex (compare figs. 6 and 7). Careful examination of the sections leads to the conclusion that probably more actual tissue growth occurred in the cortex than in the medulla, but this observation lacks adequate proof.

. That the testicular medulla invariably shows a greater capacity for growth than the ovarian medulla is ascribed to the fact that the former is in a well-developed state as the chief component of the testis, while both types of ovarian

medulla are present in a regressing condition under the domination of the cortex and hence are less capable of active growth.

The objection may be raised that the younger group contains an insufficient number of male animals whereas the older group is lacking in female animals; however, the ratios derived from gonad measurements of the two groups tend to supplement one another, and a comparison of the data leads to the same general conclusions.

D. Microscopic observations on the gonoducts and mesonephroi

1. *The younger group.* a. *The control males.* The mesonephros consists chiefly of tubules and connective tissue, although an occasional glomerulus is present, particularly in the caudal end of the organ. The wolffian duct is readily apparent, together with an occasional collecting tubule emptying into it. The duct is lined with simple cuboidal epithelium. The mesonephros is slightly separated from the gonad, except at the extreme posterior end of the testis. Some of the tubules of the mesonephros project into the gonadal tissue. However, in the more anterior region of the gonad, there is little evidence of any connection between gonad and mesonephros except by loose strands of fascia; the development of a rete connection between the gonad and the mesonephros is limited at this stage.

b. *The experimental male.* The mesonephros has been further reduced toward its ultimate conversion into an epididymis. Mesonephric glomeruli are not to be seen in the experimental animal, and the tubules are more closely compacted, with the result that the organ is now very flat. These changes are particularly evident in the area about the wolffian duct.

c. *The control females.* As in the males of this age, the mesonephros is reduced to a flattened layer containing numerous tubules but only an occasional glomerulus. The wolffian duct is recognizable as such in the caudal portion of the mesonephros. The müllerian duct is a well-developed structure

lying lateral to the mesonephros and supported by a wide mesosalpinx which is continuous with the peritoneum investing the exposed surface of the mesonephros. The walls are composed of connective tissue; smooth muscle fibers could not be recognized with certainty. The lumen of the duct is lined with a simple columnar epithelium and presents a smooth oval outline in cross section.

d. The experimental females. No gross increase in size is evident in the sections of the müllerian ducts of the experimental females. This supports the observations at autopsy, in which an increase in length and convolution, but not in diameter, of the ducts was noted. However, the simple columnar epithelial cells lining the lumen of the oviducts have become taller and more turgid so that the cells are crowded together.

As in the experimental male, the mesonephroi of the injected females are less active than those of the control animals. Glomeruli are seldom seen, and the organ is much reduced in size. The wolffian duct can be recognized in the posterior portion of the wolffian body.

2. *The older group.* a. The control males. The mesonephros is well separated by a thickened fibrous layer from the testis. No glomeruli are present, and the mesonephros consists only of collecting tubules and a wolffian duct embedded in heavy connective tissue, forecasting the ultimate conversion of the wolffian body into an epididymis. Rete cords are very infrequent.

b. The experimental males. The wolffian body shows somewhat further regression than in the control males (fig. 5). It is well separated from the testis by the intervening tunica albuginea, which overlies the entire surface of the gonad. There are still few signs of rete connections, although the mesonephric and seminiferous tubules are in a few cases in very close proximity. The wolffian duct is large and easily recognized in the median and posterior portions of the mesonephros (fig. 5). The müllerian duct, as in the controls, was not observed.

c. The control females. The mesonephros resembles that of a control male of this age (fig. 6). The oviduct is similar to, but proportionately larger than, that of a control female of the younger group.

d. The experimental females. The mesonephros presents the same picture of regression as that already described for this age group (fig. 7). The oviduct is markedly hypertrophied, as evidenced by greater cross section area and by the increased length and convolution observed at autopsy. The epithelial cells lining the oviducal lumen are turgid and thrown into folds, evidence of a rapid and unequal growth in response to stimulation (fig. 7).

DISCUSSION

Riddle and Flemion ('28), using immature doves as test animals, found that the implantation of mature dove anterior pituitaries or the intraperitoneal injection of a glycerin extract of the anterior pituitaries of cattle resulted in a marked hypertrophy of the testes, whereas the ovaries showed little stimulation. These results were essentially confirmed by the studies of Riddle and Polhemus ('31) on the immature pigeon and of Schockaert ('31) on the immature male duck. Similarly, Burns ('30, '34) and Burns and Buyse ('31, '33, '34) showed in several experiments with *Amblystoma tigrinum* larvae that both homeotransplantation of adult pituitaries and the injection of mammalian anterior lobe extract were followed in the males by a striking hypertrophy of the testes and concomitant development of accessory sex structures, whereas the females showed little response, the ovaries being at best only slightly stimulated while the accessories were unaffected. Similar experimental treatment of recently metamorphosed but sexually immature *A. tigrinum* elicited a definite response from the females in that incomplete ovogenesis and some degree of oviduct development were observed; the males, however, exhibited much greater gonad hypertrophy, as well as spermatogenesis and growth of accessory structures.

It has been shown that, relatively and absolutely, the testis of the immature alligator undergoes greater hypertrophy than the ovary following the administration of hypophysis extract (fig. 1 and table 1). This sexual difference in gonad response agrees with that observed in birds and amphibians and is in keeping with the close phylogenetic relationship of these vertebrate classes to the reptiles.

Smith ('26) was the first to demonstrate that the precocious development of the accessory sex structures in an immature female mammal (rat) following hypophyseal stimulation is due to the secretory activity of the animal's ovaries since, when the experiment was performed with ovariectomized animals, the accessory structures did not show precocious maturity, but rather became atrophic. Smith's observations have been repeatedly confirmed. The hypertrophy of the oviducts of the injected female alligators is ascribed to the inception of, or to an increase in, the elaboration of a female sex hormone by the ovaries (figs. 6, 7).

Repeated experiments have shown that it is possible to induce precocious spermatogenesis not only in immature male amphibians, as was indicated above, but also in young mammals and birds. The absence of spermatogenesis in the immature alligators, particularly in those of the older series, may perhaps be due to a failure to continue the course of injections for a sufficiently extended period of time. It also seems probable that spermatogenesis could be induced more easily in animals which were less far removed in age from the normal onset of sexual maturity.

SUMMARY

1. Injections of alkaline extract of whole sheep hypophysis into sexually immature alligators, beginning at 4 months after hatching, resulted in a marked hypertrophy of the testes, with a lesser but significant growth of the ovaries. The oviducts were increased in length and diameter as a result of the increased secretion of female sex hormone from the stimulated ovaries; the wolffian ducts did not respond.

2. Injections of larger quantities of the same hormone into 18-month-old animals resulted in an even further acceleration of sexual development. Testicular medulla showed much greater hypertrophy (1:27.47) than did ovarian cortex (1:1.96), although the average volume of a control testis was exceeded by the volume of the single control ovary (1:4.14). In keeping with the precocious approach to sexual maturity, the oviducts showed a marked increase in length and diameter, the wolffian ducts were not affected, and the mesonephroi of the males had undergone further reduction toward epididymides as a result of experimental treatment.

3. Spermatogenesis was not induced in either group of injected animals; this situation was probably due either to the relatively immature condition of the subjects or to failure to continue the experimental treatment for a sufficient period of time.

LITERATURE CITED

- BURNS, R. K., JR. 1930 Effects of hypophyseal hormones upon *Amblystoma* larvae, following transplantation or injection, with special reference to the gonads. *Proc. Soc. Exp. Biol. and Med.*, vol. 27, p. 836.
- 1934 The transplantation of the adult hypophysis into young salamander larvae. *Anat. Rec.*, vol. 58, p. 415.
- BURNS, R. K., JR., AND ADRIAN BUYSE 1931 The effects of extracts of the mammalian hypophysis upon immature salamanders. *Anat. Rec.*, vol. 51, p. 155.
- 1933 The induction of precocious maturity in the reproductive tract of recently metamorphosed female salamanders by an extract of the mammalian hypophysis. *Anat. Rec.*, vol. 58, p. 37.
- 1934 The effect of an extract of the mammalian hypophysis upon the reproductive system of immature male salamanders after metamorphosis. *J. Exp. Zool.*, vol. 67, p. 115.
- CLAUSEN, H. J. 1935 The effects of ovariectomy and hypophysectomy on parturition in snakes. *Anat. Rec.*, vol. 64 (Suppl.), p. 88.
- CUNNINGHAM, J. T., AND W. A. M. SMART 1934 The structure and origin of corpora lutea in some of the lower vertebrata. *Proc. Roy. Soc., B*, vol. 116, p. 258.
- EVANS, L. T. 1935 a The effects of Antuitrin S and sheep pituitary extract on the female lizard, *Anolis carolinensis*. *Biol. Bull.*, vol. 68, p. 355.
- 1935 b The effect of Antuitrin S on the male lizard, *Anolis carolinensis*. *Anat. Rec.*, vol. 62, p. 213.
- FORBES, T. R. 1934 Effect of injections of pituitary whole gland extract on immature alligator. *Proc. Soc. Exp. Biol. and Med.*, vol. 31, p. 1129.

- HERLANT, M. 1933 Recherches histologiques et expérimentales sur les variations cycliques du Testicule et des Caractères Sexuels secondaires chez les Reptiles. Arch. de Biol., T. 44, p. 347.
- HOUSSAY, B. A. 1930 Accion sexual en los peces y reptiles. Rev. Soc. Argent. Biol., vol. 6, p. 686.
- KEHL, R. 1935 Note préliminaire sur le cycle génital chez quelques reptiles sahariens. C. R. Soc. de Biol., T. 118, p. 1077.
- McILHENNY, E. A. 1935 The Alligator's Life History, p. 64. The Christopher Publishing House, Boston.
- MELLISH, C. H. 1936 The effects of anterior pituitary extract and certain environmental conditions on the genital system of the horned lizard (*Phrynosoma cornutum*, Harlan). Anat. Rec., vol. 67, p. 23.
- REESE, A. M. 1915 The Alligator and its Allies, p. 17. G. P. Putnam's Sons, New York.
- RIDDLE, O., AND F. FLEMION 1928 Studies on the physiology of reproduction in birds. XXVI. The role of the anterior pituitary in hastening sexual maturity in ring doves. Am. J. Physiol., vol. 87, p. 110.
- RIDDLE, O., AND I. POLHEMUS 1931 Studies on the physiology of reproduction in birds. XXXI. Effects of anterior pituitary hormones on the gonads and other organ weights in the pigeon. Am. J. Physiol., vol. 98, p. 121.
- SCHAEFER, W. H. 1933 Hypophysectomy and thyroidectomy of snakes. Proc. Soc. Exp. Biol. and Med., vol. 30, p. 1363.
- SCHOCKAERT, J. A. 1931 Response of the male genital system of the immature domestic duck to injections of anterior-pituitary substances. Anat. Rec., vol. 50, p. 381.
- SMITH, P. E. 1926 Hastening development of female genital system by daily homeoplastic pituitary transplants. Proc. Soc. Exp. Biol. and Med., vol. 24, p. 131.
- TURNER, C. D. 1935 The effects of Antuitrin-S on the male genital organs of the lizard (*Eumeces laticeps*) during seasonal atrophy. Biol. Bull., vol. 69, p. 143.

PLATE 1

EXPLANATION OF FIGURES

2 A ventral view of the gonads and gonoducts of a control male (left) and an experimental male (right) of the older series. Note the remarkable hypertrophy of the testes following hormonal stimulation. The vasa deferentia are visible as paired tubules extending posteriorly from the testes to the stump of the cloaca, most of which has been resected. Part of the anus of the control male may be seen below. $\times 1\frac{1}{2}$.

3 The ovaries and oviducts of a control female (left) and an experimental female (right) of the older series. Both ovaries and ducts show a striking hypertrophy as a result of experimental treatment. The latter structures, seen as convoluted tubes lying just lateral and extending posterior to the gonads, have increased in both length and diameter. The metanephroi lie dorsal to the darkly pigmented peritoneum. $\times 1\frac{1}{2}$.

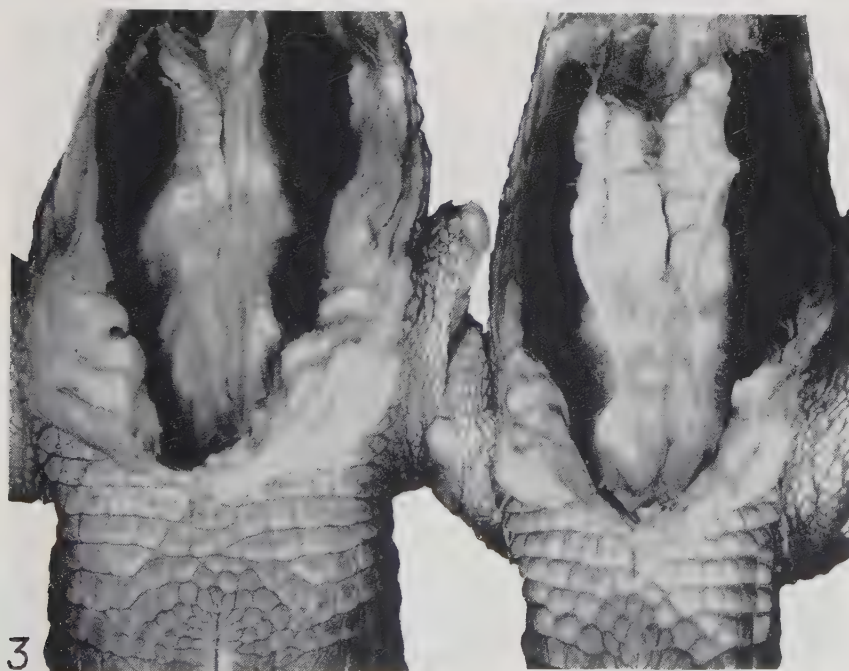
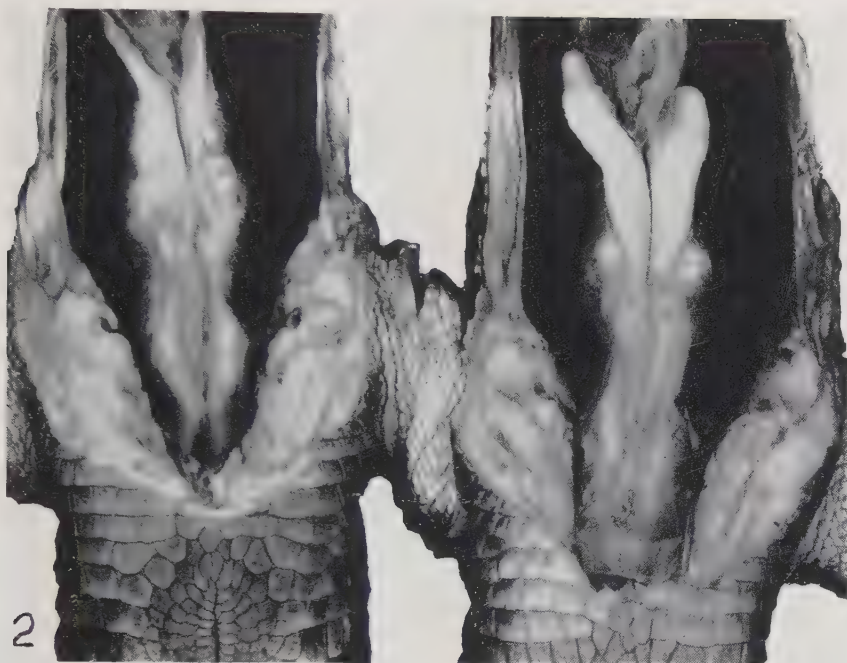


PLATE 2

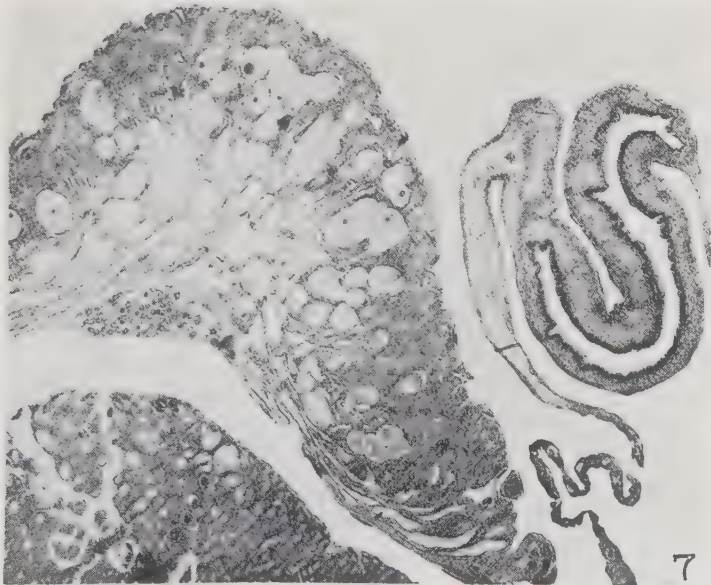
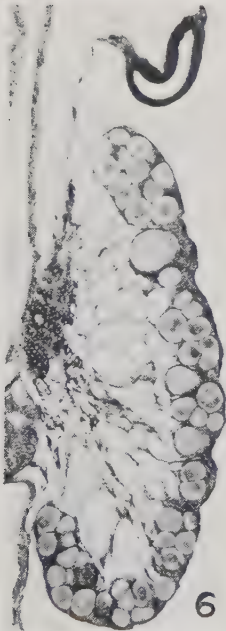
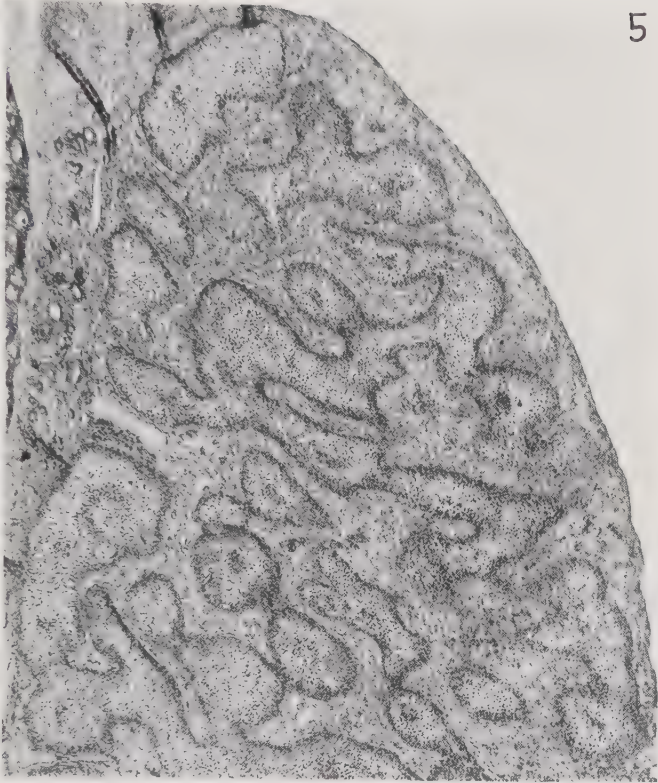
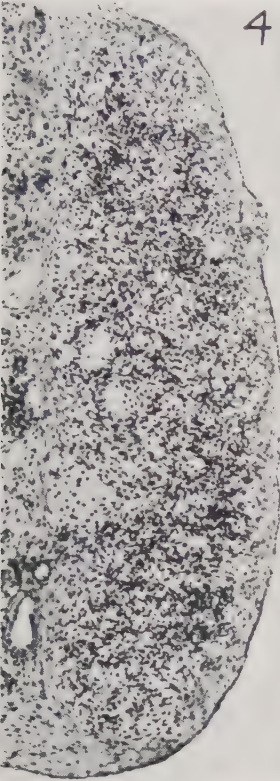
EXPLANATION OF FIGURES

4 A section of control testis such as is seen in the older series of alligators. Note the fungiform area of cortex (upper right) penetrating the dense tunica albuginea. Primitive seminiferous tubules are evident in the medulla. To the left are seen small adjacent areas of mesonephros and adrenal. $\times 100$.

5 Part of a typical section through an experimental testis of the older series. Note the tubules crowded with germ cells and the dense fibrous tunica albuginea and septula testis. The mesonephros and wolffian duct are seen at the left. $\times 50$.

6 A section of ovary and oviduct similar to those of the control females of the older series. Note the cortex crowded with developing germ cells. The mesosalpinx arises just lateral to the regressing mesonephros, seen at the left. $\times 25$.

7 An experimental ovary and oviduct of the older series. The cortex has grown so rapidly as to draw taut the medullary strands. Hypertrophy of the oviduct is evident. The mesonephros (left of center) lies dorsal to the ovary. Below is seen a portion of the metanephros. $\times 25$.



THE OXYDASE REACTIONS AS APPLIED TO THE MEGAKARYOCYTE AND BLOOD PLATELET OF THE RAT

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Whether blood platelets originate from lymphocytes, monocytes, granular leucocytes or cells of the reticulo-endothelial system as well as from megakaryocytes is still an open question. The observations of Wright first made in 1906 and published again in 1910 regarding the latter as progenitors of platelets have been many times corroborated. The azurophilic granules of both platelets and megakaryocytes as described by him have been the structural characteristics most often emphasized. Further investigation of the granules has brought little positive knowledge as to their nature. As is well known they stain supravitaly with neutral red and brilliant cresyl blue. Barta ('32) finds that after methyl alcohol fixation the granules are unstained in 1% eosin, erythrosin or acid violet but that they are colored in Loeffler's methylene blue, methyl violet and toluidin blue. Roskin and Grinbaum ('26) and Beams and King ('33) report that they do not react to Feulgen's nuclear stain, and Corradetti ('34) finds that they are the only structures of the blood cells to withstand treatment with hydrochloric acid. Kingsley ('35, '37) has recently described them as specific granules by which he is able to differentiate even the early stages of the megakaryocytes from surrounding cells.

¹ Appreciative acknowledgment is made to E. Quamme and J. Hastings for certain technical details used in the various methods.

In the present study it was found that the azurophilic granules of both platelets and megakaryocytes were easily and clearly demonstrated with a combination of Wright's and Giemsa's stains using Isaac's technique for the marrow. The granules were uniformly negative in the oxydase and peroxydase tests. This last is contrary to findings reported in 1935 by Smith and Hastings.

METHODS

After comparing ordinary smears of bone marrow on slides with others of cells suspended in plasma according to Isaacs ('28, '30) it was decided to use the latter method. By this procedure all of the cells of the marrow were better preserved and more deeply stained. And what was important for this study, it was possible by this method to have both megakaryocytes and platelets in the same medium giving a chance for more direct comparison of staining reactions. It was found that the most even and best results were obtained after the suspension had stood 20 minutes. As a further precaution the smears of bone marrow suspensions and peripheral blood were made on two halves of the same slide. Platelets were also studied in smears of human blood. In the examination of platelets in blood smears care was taken to make decisions only on those which could be clearly seen.

All glassware was cleaned according to the methods outlined for supravital technique (Sabin, '23) and pipettes and centrifuge tubes were coated with paraffin. Heparin was used as the anticoagulant in obtaining the plasma. The oxydase reactions were always started within at least 2 hours after making the preparations.

OBSERVATIONS AND DISCUSSION

The azurophilic granules of the megakaryocytes and blood platelets did not react in the oxydase and peroxydase tests which were used in this study (table 1) although the results were positive with the specific granules of the neutrophils and eosinophiles and varying numbers of bodies within the

monocytes. Whether these latter were exogenous or endogenous could not be determined. An examination of the basophiles was not made. Table 3 shows that while most of the previous investigators found these granules similarly inactive,

TABLE 1

Summary of methods used in the study of the oxydase and peroxydase reactions of megakaryocytes and blood platelets

AUTHOR	REAGENTS	COUNTERSTAIN	COLOR OF OXYDASE GRANULES
Block and Peck	Dopa	Safranin	Dark brown to black
Lambright	Alpha naphthol, hydrogen peroxide	Methylene blue	Blue to red
McJunkin	Benzidine, hydrogen peroxide	Wright's	Amber brown
Sato and Yoshimatsu	Copper sulphate, benzidine, hydrogen peroxide	Safranin	Blue green
Washburn (Kolmer and Boerner)	Nitroprusside, benzidine, hydrogen peroxide	Wright's	Black

TABLE 2

The oxydase and peroxydase tests as applied to the megakaryocyte and blood platelet

METHOD	HUMAN BLOOD PLATELETS	RAT PERIPHERAL BLOOD PLATELETS	RAT BONE MARROW MEGAKARYOCYTES
Block and Peck	—	—	—
Lambright	—	—	—
McJunkin	—	—	—
Sato and Yoshimatsu	—	—	—
Washburn (Kolmer and Boerner)	—	—	—

two report positive staining. Contrary to the findings of Roskin and Grinbaum ('26), with us a successful dopa reaction revealed unstained megakaryocytes surrounded by strikingly blackened cells of the granular series. Because of the precipitate the blood platelets were more difficult to see,

but with a safranin counterstain they could be definitely ascertained. Katsunuma ('26) described a positive oxydase reaction in the blood platelets and megakaryocytes which was difficult to find in normal blood but quite clear in myeloid leukaemia, fetal blood and in experimental anemia in rabbits. He believed the oxydase granules corresponded to the azurophilic ones and gave the positive reaction as a microchemical support to Wright's findings. However, Barta ('32) believed

TABLE 3

History of the oxydase and peroxydase reactions applied to megakaryocytes and blood platelets

INVESTIGATOR	YEAR	METHOD	MEGAKARYOCYTES	PLATELETS
Graham	1916	Alpha-naphthol, hydrogen peroxide		—
	1918	Benzidine, hydrogen peroxide		—
Richter	1925	McJunkin	—	—
Katsunuma	1926	Gierke, indophenol oxydase	+	+
Roskin and Grinbaum	1926	Dopa,	+	+
		Graham benzidine	—	—
Knoll	1927	Alpha-naphthol, dimethylparaphenyl- endiamibase	—	
Barta	1932	Schulze, Graham	—	—

that the positive oxydase reactions of megakaryocytes in infectious diseases were due to phagocytosed material and therefore not specific differentiations of the cell.

That blood platelets may be derived from cells other than the megakaryocytes is believed by many, though hard to prove. In the study by Birch ('30) on the origin of pseudothrombocytes from leucocytes, she makes the statement that "the possibility exists that leukoplastids may be normal constituents of the human blood." According to her these leukoplastids may be either granular in origin, arising from neutrophils, eosinophiles, or basophiles, or non-granular, arising

from lymphocytes, lymphoblasts, or myeloblasts. Of these sources only the specific granules of neutrophiles and eosinophiles were found to have clear-cut peroxidase reactions in these studies and table 2 indicates that in normal blood (human and rat), at least, platelets are not so derived. In the peripheral blood of the rat basophiles appear to be absent. In the case of two neutrophiles in fixed smears, pseudopods full of oxydase granules were seen and looked as though they were in the process of being pinched off. No such pieces with clear oxydase granules were seen free among the cells.

SUMMARY

1. The azurophilic granules of the megakaryocyte stain more deeply and easily in cells suspended in plasma (Isaacs' method) than in ordinary smears.

2. The azurophilic granules of normal human blood platelets and of normal rat platelets and megakaryocytes give negative oxydase and peroxidase reactions.

3. In normal blood there is no evidence that any platelets are derived from neutrophiles or eosinophiles.

LITERATURE CITED

- BARTA, I. 1932 Über Bau und Funktion der Megakaryozyten. *Folia Haemat.*, vol. 47, p. 168.
- BEAMS, H. W., AND R. L. KING 1933 Feulgen's 'nuclearreaktion' and blood platelets. *Anat. Rec.*, vol. 55 (suppl.), p. 46.
- BIRCH, C. L. 1930 The origin of pseudothrombocytes from leucocytes. *Anat. Rec.*, vol. 46, p. 45.
- BLOCK, B., AND S. M. PECK 1930 Der Nachweis der Oxydase in den Zellen des myeloischen Systems durch 3,4-Dioxyphenylalanin. *Folia Haemat.*, vol. 41, p. 166.
- CORRADETTI, A. 1934 Ricerche microchimiche sui megacariociti e sulle piastrine. *Haemat.*, vol. 15, p. 207.
- GRAHAM, G. S. 1916 The oxidizing ferment of the myelocyte series of cells and its demonstration by an alpha-naphthol-pyronin method. *J. Med. Research*, vol. 35, p. 231.
- 1918-1919 Benzidine as a peroxidase reagent for blood smears and tissues. *J. Med. Research*, vol. 39, p. 15.
- ISAACS, R. 1928 Alterations of tissue cells in the blood stream. *Science*, vol. 68, p. 547.
- 1930 The physiologic histology of the bone marrow. *Folia Haemat.*, vol. 40, p. 395.

- KATSUNUMA, S. 1926 Genese der Blut-plättchen im Lichte der Oxydasereaktion. *Folia Haemat.*, vol. 32, p. 29.
- KINGSLEY, D. M. 1935 The development of the megakaryocyte in the pig. *Anat. Rec.*, vol. 61 (suppl.), p. 29.
- 1937 A technic for megakaryocytes and platelets in sections. *Folia Haemat.*, vol. 57, p. 87.
- KNOLL, W. 1927 Blut und blutbildende Organe menschlicher Embryonen. *Denkschriften der schweizerischen naturforschenden Gesellschaft. Mém. de la Soc. Helvétique des Sci. Nat.*, vols. 62-64, p. 1.
- KOLMER, J. A., AND F. BOERNER 1931 Approved laboratory technique, p. 89. D. Appleton and Co., New York.
- LAMBRIGHT, G. L. 1921 Leukemia: type diagnosis by oxydase method of blood staining. *Am. J. Med. Sci.*, vol. 161, p. 209.
- McJUNKIN, F. A. 1920 A benzidin-polychrome stain for blood. *J. Am. Med. Assoc.*, vol. 74, p. 17.
- RICHTER, M. N. 1925 Leukemia. The relative values of cell morphology and the peroxydase reaction as diagnostic aids. *Arch. Int. Med.*, vol. 36, p. 13.
- ROSKIN, G. O., AND F. T. GRINBAUM 1926 A contribution to the knowledge of blood platelets. *Arb. Microbiol. Inst. (Moscow)*, vol. 2, p. 137. *Biol. Abstracts*, vol. 2, p. 1577.
- SABIN, F. 1923 Studies of living human blood-cells. *Johns Hopkins Hosp. Bull.*, vol. 34, p. 277.
- SATO, A., AND S. YOSHIMATSU 1925 The peroxidase reaction in epidemic encephalitis. *Am. J. Dis. Child.*, vol. 29, p. 301.
- SMITH, C., AND J. HASTINGS 1935 A study of the megakaryocyte and blood platelet in the rat. *Anat. Rec.*, vol. 64 (suppl. no. 1), p. 95.
- WRIGHT, J. H. 1910 The histogenesis of the blood platelets. *J. Morph.*, vol. 21, p. 263.

FUNCTIONAL HOMEOPLASTIC GRAFTS OF THE ADRENAL GLAND OF NEWBORN RATS

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FIVE FIGURES

Autotransplantation of the adrenal gland has been successfully performed on small animals, especially rats (Jaffe, '26; Poll, 1898; Wyman and tum Suden, '32) and guinea pigs (Jaffe, '27, '27 a), but results have been uniformly unsuccessful with dogs (Blodinger, Klebanoff and Laurens, '26; Johnson and Johnson, '31; Oldberg, '29). Surviving cortical grafts of adrenal glands of rabbits have been reported (Busch, Leonard and Wright, '08). For review of the work on transplantation, attention is directed to the articles of Jaffe ('27), Britton ('30), and Loeb ('30).

Homeotransplantation of the gland has been less successful than autotransplantation. Schmieden ('03) reported some success with rabbits and Jaffe ('27), working with rats, reported four successful homeografts out of fifteen trials. Wyman and tum Suden ('32) secured successful homeografts when they exchanged glands between litter mates. They showed that such homeografts grew equally as well as autografts. In a large series of homeografts between unrelated rats of our own strain we (Ingle, Higgins and Nilson, unpublished data) found that only an occasional graft survived and functioned. If, on the other hand, glands were exchanged between rats of a strain closely inbred for many generations, successful homeografts were obtained in a high percentage of the animals (Ingle, Higgins and Nilson, unpublished data).

Lux, Higgins and Mann ('37) attempted to acclimatize adrenal glands of newborn rats to unrelated adult rats by growing fragments of the glands in media containing the body fluids of the animals selected as hosts. After the tissue had grown *in vitro* for 2 weeks, the fragments were grafted to the adrenalectomized hosts. Successful grafts were obtained in half of their grafted animals but the data were not convincing that any adaptation of the biologic constituents of the grafts to those of the hosts was made by 'in vitro' growth of the glands prior to transplantation. They concluded that successful grafts were owing to similarities between the genetic constitutions of the donors and those of the hosts rather than to changes in the tissue during the growth *in vitro*.

Because of the success of Lux, Higgins and Mann ('37), with adrenal glands of newborn rats, we grafted adrenal glands of newborn rats to unrelated adults without first growing them in tissue culture media. A number of attempts have been made to graft embryonic tissues or tissues of newborn rats, and a review of these studies was given by May ('33). May has reported successful homeografts of the thyroid and parathyroid glands of newborn rats, grafted to the anterior chamber of the eye. May ('35) likewise grafted the pituitary bodies of newborn rats and secured successful grafts. Salmon and Severinghaus ('36) likewise have reported successful results with homeografts of thyroid glands of newborn rats. Considerably after our work had begun, Rutishauser and Guye ('36) reported that they secured viable homeografts of the adrenal glands of newborn rats, grafted to the renal parenchyma of adult rats. However, percentages of surviving functional grafts were not included in their report.

METHODS AND PROCEDURES

The adrenal glands were removed from newborn rats and immediately placed in the fascia along the femoral veins of unrelated rats. Ether anesthesia was used, and satisfactory surgical technic was employed. One group of forty healthy, male, adult rats, previously adrenalectomized, were used as

hosts for grafts. In another group, consisting of ten healthy adult, male rats, the same procedure of grafting was followed and the same kind of material was grafted but these recipient animals were not adrenalectomized. Of a third group, consisting of forty-five healthy adult male, previously adrenalectomized rats, fifteen received adrenal glands removed from rats 2 weeks old; fifteen, adrenal glands from animals 1 month old and fifteen, adrenal glands from animals 2 months old.

The basic diet of the hosts was our regular, standard ration. Following transplantation, all adrenalectomized rats, that is, rats of the first and third groups, were given 0.8% sodium chloride in their drinking water and were fed a ration containing 1.5% sodium chloride. Rats of the first and third groups received the added sodium chloride for 1 week after transplantation.

In the first group, five rats were killed at intervals in the first five weeks following grafting, to determine the histologic appearance of the graft. All other grafted rats of this group which lived 5 months were explored and the adrenal tissue was removed from the groins. Duration of life of each animal following removal of the grafted glands was noted. All grafts were fixed, sectioned and stained with hematoxylin and eosin, with Mallory's connective tissue stain, and for fat and chromaffin reaction. Of the second group of ten adult rats, two were explored each week for 5 weeks. The grafts were removed, fixed, sectioned and stained. Concerning the third group, careful data were kept on the length of time the animals lived and the number of functional grafts recovered.

RESULTS

Of the forty rats comprising the first group, twenty-four survived with functional cortical grafts in the groin. Of these twenty-four animals, five were killed during the first 5 weeks after transplanting, as has been said, and nineteen lived for 5 months. Each of the nineteen had a cluster of small cortical nodules in the groin closely adjacent to the femoral vessels (fig. 1). The number and size of these nodules

varied. Although ten small glands were grafted, yet the number recovered never exceeded seven. The size of these cortical nodules bore some relationship to the number that regenerated and survived. If three or four survived, the size of each approximated that of a normal gland but if more survived, the size of each nodule was correspondingly reduced. The total amount of restored cortical tissue was slightly in excess of the combined weight of the adrenal glands that had been removed from the host. Sixteen of the forty animals



Fig. 1 Cluster of adrenal glands in groin of host, 5 months after grafting. $\times 5$.

which were grafted with glands from newborn rats died of adrenal insufficiency within 2 to 3 weeks after the salt content of their drinking water and ration had been reduced to normal. The grafts in these animals had failed to survive. All nineteen animals which had viable grafts after 5 months died within 3 weeks after grafts had been removed from the groin, indicating that they were functioning and delivering to the organism the active cortical principle.

In the second group, the grafts which were removed at weekly intervals from the groins of rats, which had not been

adrenalectomized, were always necrotic. We did not find a functioning graft in this entire series. Soon after transplanting they were infiltrated with leukocytes and were either resorbed or fibrotic when the last pair of animals were killed at 5 weeks.

Observations on the third group of adult animals were continued for 10 weeks after the salt content of their drinking water and ration had been reduced. There was a considerable difference in the number of animals which survived in each of the three subgroups. Of the fifteen adult rats grafted with glands removed from unrelated rats 2 months old, only one animal survived for the 10 weeks with a mass of functioning cortical tissue. Of the fifteen grafted with glands of rats 1 month old, three were alive, with functioning grafts at 10 weeks. Of the fifteen grafted with glands of rats 2 weeks old, seven were alive and had functional homeografts at 10 weeks. When contrasting these figures with those given above, wherein twenty-four of the forty rats grafted with adrenals of newborn rats survived with functional grafts, it seems clear that age of the transplanted tissue is a factor in the survival of a homeograft.

OBSERVATIONS ON THE GRAFTS

When an adrenal gland of a newborn rat (fig. 2) was removed and grafted to the groin of an adult, adrenalectomized rat, it degenerated exactly in the manner of an autografted adult gland (Pencharz, Olmsted and Giragossintz, '31). Necrosis first appeared in the center of the gland, the medulla was destroyed and gradually the entire gland, except for a narrow margin of the glomerular zone, just below the capsule, degenerated (fig. 3). During this time blood vessels penetrated through the capsule from adjacent tissues and polymorphonuclear leukocytes invaded the necrotic portion. Subsequently, regeneration of new cortical cells took place. This occurred not only from cells in the persistent portion of the glomerular zone but from the adjacent capsule as well. This is in accord with Zwemer's ('36) finding that regeneration of

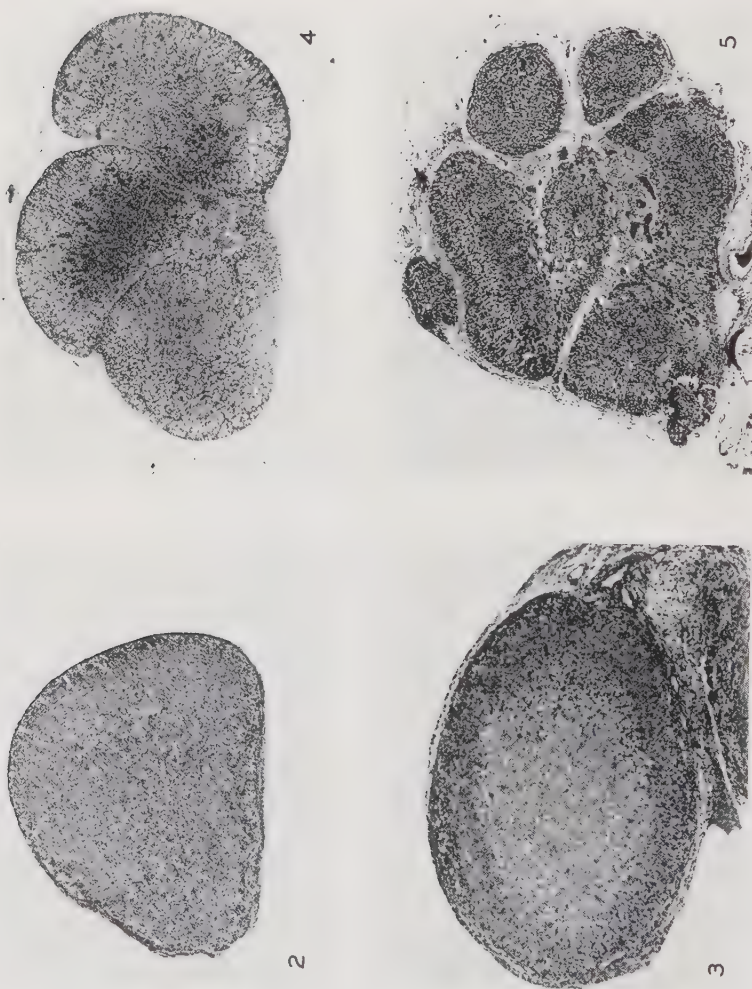


Fig. 2 Section of adrenal gland of newborn rat. Hematoxylin and eosin. $\times 60$.
 Fig. 3 Section of gland removed from groin 72 hours after grafting. Hematoxylin and eosin. $\times 60$.

Fig. 4 Section of cortical mass removed from groin 1 month after grafting. Hematoxylin and eosin. $\times 40$.

Fig. 5 Section of cortical mass removed from groin 5 months after grafting glands of newborn animals. Seven glands survived. Hematoxylin and eosin. $\times 25$.

zona glomerulosa takes place from the capsule in a normal adrenal gland.

Regeneration of cortical tissue proceeded from the capsule and extended into the center of the gland as rapidly as the old necrotic tissue was removed. Sections of grafts removed at 1 month showed evidence of considerable regeneration of cortical tissue, with some necrotic material still persisting (fig. 4). In sections of grafts removed 5 months after grafting were found vascularized masses of cortical tissue comprised of smaller nodules which had regenerated from the separate capsules of the grafted glands (fig. 5).

The characteristic cellular response of the host against a homeograft occurred in the sixteen animals which died of adrenal insufficiency soon after grafting. Lymphocytes infiltrated the gland and foreign body giant cells were commonly seen. Here were host reactions against a graft such as Loeb ('30) has described.

COMMENT

The adrenal gland of the newborn rat may be grafted to an unrelated adult rat and a large percentage of successful functional grafts may survive. Our data show that the results were somewhat better than when the technic of tissue culture was employed to acclimatize the adrenal fragments of one animal to the body fluids of the host before grafting. It seems, therefore, that the successful grafts obtained by Lux, Higgins and Mann ('37) were owing to the fact that they were grafting undifferentiated tissue of newborn animals rather than to changes which they may have induced in the biologic differentials of the grafts by growing them in host media.

The chance of securing successful homeografts were greater when tissues of newborn animals were used. It seems probable that the age and the degree of differentiation attained by a grafted tissue may be factors which control or modify its survival and function in the host. In our experience, the older the donor from which a gland to be grafted was removed, the less likely were we to secure viable grafts.

Homeografts of an adrenal gland of an adult only occasionally survived; and yet of forty homeografts of adrenal glands of newborn animals, 60% survived and functioned. But age and degree of differentiation of a gland do not constitute all that is involved in its survival and function. Of forty animals which received grafts of adrenal glands of newborn rats, as stated above, sixteen died. The grafts in all of these had been destroyed by an aggressive cytologic reaction of the host. Since all grafts in these sixteen hosts were placed along the femoral vein in exactly the same way as that employed in placing the grafts in the entire group, failure of the grafts to survive cannot have been owing to mechanical or to operative procedures. Furthermore, the age of the donors could not have been cause for failure since all sixteen were grafted with glands removed from donors, of the same age as those from whom were taken grafts which survived; that is the grafts were removed at birth or very soon thereafter.

We believe that our failures were owing to marked biologic or genetic differences between the donors and the hosts. As far as we know there was only a remote relationship between the donors providing the glands and the adults which received them. All were taken at random from our colony, which is not closely inbred, but which has been developed from a stock derived from the Wistar strain some time ago. It seems likely that some of the donors may have resembled, genetically more closely than others, the hosts to which their adrenal glands were grafted. Likewise, others may have been genetically unlike the hosts. And it is likely that viability of grafts depended entirely on the degree of the differences that existed between the genetic constitutions of those animals serving as hosts and those serving as donors. We are repeating this experiment, using rats of a closely inbred strain in which brother to sister matings have been made for thirty-eight generations.

CONCLUSIONS

1. Adrenal glands of newborn animals, when grafted to adult animals, will regenerate, grow and function in a high percentage of cases.

2. Regeneration of cortical tissue proceeds from the capsule, but medulla does not survive nor regenerate.

3. A considerable percentage of such grafts will not survive. Host reactions against these grafts resemble the well-known reaction against homeografts.

4. The success or failure of a graft probably depends on the degree of genetic identity between donor and host.

5. Glands of newborn animals will not regenerate, to any extent, in the presence of the host's own adrenal glands.

6. It seems apparent that another factor in successful homeotransplantation is the age of the donor; the younger the donor the greater is the likelihood of survival of the graft.

LITERATURE CITED

- BLODINGER, I., H. E. KLEBANOFF AND HENRY LAURENS 1926 Suprarenal transplantation in the dog. *Am. J. Physiol.*, vol. 76, pp. 151-157.
- BRITTON, S. W. 1930 Adrenal insufficiency and related considerations. *Physiol. Rev.*, vol. 10, pp. 617-682.
- BUSCH, F. C., T. M. LEONARD AND T. WRIGHT 1908 Further results in suprarenal transplantations. *J. Am. Med. Assn.*, vol. 51, pp. 640-642.
- INGLE, D. J., G. M. HIGGINS AND H. W. NILSON Unpublished data.
- JAFFE, H. L. 1927 On the transplantation of the guinea pig suprarenal and the functioning of the grafts. *J. Exp. Med.*, vol. 45, pp. 587-594.
- 1927 a The suprarenal gland. *Arch. Path.*, vol. 3, pp. 414-453.
- JAFFE, H. L., AND ALEXANDRA PLAVSKA 1926 Functioning autoplasic suprarenal transplants. *Proc. Soc. Exp. Biol. and Med.*, vol. 23, pp. 528-530.
- JOHNSON, ADELAIDE AND VICTOR JOHNSON 1931 Attempted autotransplantation of the adrenal cortex. *Am. J. Physiol.*, vol. 97, pp. 392-395.
- LOEB, L. 1930 Transplantation and individuality. *Physiol. Rev.*, vol. 10, pp. 547-616.
- LUX, LYDIA, G. M. HIGGINS AND F. C. MANN 1937 Functional homeografts of the rat adrenal grown in vitro. *Anat. Rec.*, vol. 70, pp. 29-43.
- MAY, R. M. 1933 Action vicariante durable de la greffe intraoculaire de thyroïde de raton nouveau-né sur le développement du rat blanc ethyroïde. *Arch. de Biol.*, T. 44, pp. 149-178.
- 1935 La greffe bréphoplastique de l'hypophyse chez le rat. *Compt. rend Soc. de Biol.*, T. 120, pp. 867-870.
- OLDBERG, ERIC 1929 An attempt to auto-transplant the adrenal. *Am. J. Physiol.*, vol. 91, pp. 275-283.

- PENCHARZ, R. I., J. M. D. OLMSTED AND G. GIRAGOSSINTZ 1931 The survival of rats after total and partial adrenalectomy and adrenal transplantation. *Physiol. Zool.*, vol. 4, pp. 501-514.
- POLL, H. 1898 Ueber das Schicksal der verpflanzten Nebenniere. *Centralblat. f. Physiol.*, Bd. 12, S. 321-326.
- RUTISHAUSER, ERWIN, AND PIERRE GUYE 1936 La bréphoplastie surrénalienne chez le rat. *Compt. rend. Soc. de biol.*, T. 121, pp. 1046-1049.
- SALMON, THEODORA N., AND A. E. SEVERINGHAUS 1936 Functional auto- and homoplastic thyroid grafts in the rat. *Proc. Soc. Exp. Biol. and Med.*, vol. 34, pp. 251-253.
- SCHMIEDEN, VIKTOR 1903 Erfolgreiche, experimentelle Verlagerung von Nebennierengewebe, ein Beitrag zur Lehre von den Strumae suprarenales aberratae. *Deutsch. Ztschr. f. chir.*, Bd. 70, S. 453-504.
- WYMAN, L. C., AND CAROLINE TUM SUDEN 1932 Studies on suprarenal insufficiency. XI. The growth of transplanted cortical tissue in the rat. *Am. J. Physiol.*, vol. 101, pp. 662-667.
- ZWEMER, R. L. 1936 A study of adrenal cortex morphology. *Am. J. Path.*, vol. 12, pp. 107-114.

SPERMATOGENESIS IN A SEX-REVERSED FEMALE AND IN NORMAL MALES OF THE DOMESTIC FOWL, *GALLUS DOMESTICUS*¹

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THIRTY-FOUR TEXT FIGURES AND THREE PLATES (ELEVEN FIGURES)

INTRODUCTION

Genetical studies indicate that in birds the female is heterogametic, the male homogametic with respect to the sex determining factor. Cytological investigations which were undertaken to verify this conclusion by chromosome studies show a notable lack of agreement in the observations of the different workers. Table 1 summarizes the literature.

Most of the work has been on the Gallinaceae, particularly the domestic fowl. Several papers report on the pigeon, duck and parakeet. Unger ('36) has recently investigated two species of passerine birds.

The work on chromosomes may be divided into three periods. Prior to 1923 the number of chromosomes reported for any species is small. From 1923 to 1930 the number described for the fowl falls within a range of 29 to 45, with the average about 35 to 37. Since 1930 the increase in the number of small chromosomes observed has raised the total count. Obviously these changes are due to improvement in technique as well as in accuracy of observation.

All investigators are agreed that males are homogametic, the female heterogametic. Guyer ('16) reported an unpaired element in the chromosome complex of both male and female

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TABLE 1

AUTHOR	RACE OR SPECIES	MATERIAL	CHROMOSOME NUMBER				SEX DETERMINATION		SEX CHROMOSOME
			2N	1st cyte ♀ 6 ♀	N	2nd cyte	Male	Female	
Loyez, '06	Gallus domesticus								
Sonnenbrodt, '08		Gonads, newly hatched to laying							
Guyer, '09		Adult testis	15-19 spg. prob. 17	9 ♂ 8 bival.	4.5 (incom. fusion)				Largest chromosome heterogametic in ♂
Lécaillon, '10			12 ♀ 12 somitic		No reduction				
Boring, Pearl, '14		Testis							Unable to confirm Guyer
Guyer, '16		Adult testis, embryos	18 spg.	9	4.5 (fusion in pairs)		Homo-gametic	Hetero-gametic	Large curved chromosome
Cutler, '18	Gold campine	Testis	18-20 spg.	8-10	Clumping				
Boring, '23 (Stevens)		Adult testis	32 spg.	18-22					
Crew, '23			32 or more						
Hance, '24		Embryonic gonad adult testis, tissue culture	30-34 soma.				ZZ	ZO	Largest chromosome
Shiwago, '24		Embryos, adult and young testis	32 ♀ 32 ♂				ZZ	ZW	Second largest
Hance, '26		Embryo, testis, tissue culture	Pro. 60-70 Meta. 35-40				ZZ	ZO	Largest chromosome
Akkinga, '27	Bankiva	Embryo	36-38						
	Barnevelder	Embryo	ca. 30-32						
	Ankona	Embryo	ca. 33						
	F ₁ Seiden ♀ × Bank. ♂	Embryo	42-44						
	F ₁ Bank. ♀ × Seiden ♂	Embryo	32				ZZ	ZW	Largest chromosome
Goldsmith, '28	Rhode Island Red, Leghorn	Embryos	36				ZZ	Z	Largest chromosome
Kemp, '30		Tissue culture	36-38						
Saguchi, '30		Tissue culture	29-39, pro. 42, meta. ca. 30						

	Gallus domesticus	Embryos	74 spg 73 oog.	37	37 (36 + Z)	ZZ	ZO	Smallest V-chromosome
White, '30		Embryos, adult testis smears	66 ± 2	33 ± 1		ZZ	Z	Largest chromosome
Popoff, '33	White Leghorn	Annion, Embryonic gonad	30-45 meta, 60-70 pro.			ZZ	Z	Largest chromosome
Sokolow and Trofimov, '33	White Leghorn, Minorka, R. I. Red Wyandotte, Leghorn × Orpington Wetluga × Seiden	Embryos, soma, Embryonic testis	32-71 most ca. 50			ZZ	Z	5th chromosome
Unger, '36	Italian Plymouth Rock	Embryo, testis Embryo, soma	44 61			ZZ	Z	5th chromosome
Sokolow, Tiniakow, Trofimov, '36	Several races	Embryos, Embryonic testis				ZZ	ZO	5th chromosome
Miller (present investigation)	Buff Orpington	Testis: young ♂ ovariotomized ♀	51-60	40	5-7 large, several small	ZZ	ZO	5th chromosome
Shiwago, '29	Brown Leghorn	Testis: adult ♂				ZZ	ZW	Largest chromosome
Werner, '31	Meleagris	Annion	46 ♀ soma. 46 ♂ soma.	38		ZZ	ZWw	Largest and second largest chromosomes
Sokolow, Tiniakow, Trofimov, '34	Meleagris dom.	Embryonic testis, Emb. membranes	77 ♀ 76 ♂			ZZ	Z	4th chromosome
Sokolow, Tiniakow, Trofimov, '36	M. gallopavo	Embryos	46 ♀ 49 ♂			ZZ	Z	4th chromosome
Cutler, '18	Pheasant × fowl Phasianus	Sterile male	18-20 20-22	10-11	8			
Trofimov, '33	Phasianus colchicus					ZZ	Z	4th chromosome
Sokolow, Tiniakow, Trofimov, '36	Ph. colchicus	Embryonic gonad				ZZ	Z	4th chromosome
Unger, '36	Ph. torquatus	Embryos				ZZ	Z	4th chromosome
Sokolow, Tiniakow, Trofimov, '36	Nycthemerus argenteus	Embryos Embryonic testis				ZZ	Z	4th chromosome

TABLE 1—Continued

AUTHOR	RACE OR SPECIES	MATERIAL	CHROMOSOME NUMBER				SEX DETERMINATION		SEX CHROMOSOME
			2N	N		Male	Female		
				1st cyte	2nd cyte				
Sokolow, Tiniakow, Trofimov, '36	Tetrao tetrix	Embryos Embryonic testis							5th chromosome
Tiniakow, '34	Pavo cristatus		36-58				ZZ	Z	5th chromosome
Sokolow, Tiniakow, Trofimov, '36	Pavo cristatus	Embryo, Embryonic gonads							5th chromosome
Guyer, '09	Numida meleagris domestica	Adult testis	17 spg.	9	8, 9				
Sokolow, Tiniakow, Trofimov, '36	Numida meleagris	Embryo, Embryonic gonads							4th or 5th chromosome
Werner, '27	Indian Runner Duck	Embryonic testis, membranes	77 ♀ soma. 76 ♂	38			ZZ	ZW	Largest and second largest chromosomes
Schöneberg, '13	Anas boschas								
	Aythya ferina								
	Cairina moschata	Adult testis	ca. 16 spg.	8	8				
	Lamprolaima sponsa								
	Mareca penelope								
Guyer, '02	Turtur risorius		16 spg.	8	4 (occas. 8)				
Guyer, '02	Columba livia domestica	Adult testis, Embryos	16 spg.	8	4 (occas. 8)				
Harper, '04	C. domestica	Eggs	16 cl.	8 ♀	8 ♀				
Smith, '12	Columba hybrid	Testis	ca. 16	8	4				
Oguma, '27	C. livia dom.		62 spg., som. 61 ♀ (emb.)	31	31		ZZ	ZO	Largest V-chromosome
Unger, '36	Turdus merula	Amnion, embryos, Embryonic gonad	60-85						4th chromosome
Unger, '36	Linota cannabina	Amnion, embryos, Embryonic gonad	64-85, most 68-75						5th chromosome
Crew, Lamy, '35	Melopsittacus undulatus	Embryos	50-60, ca. 52				ZZ	Z	Largest chromosome, a V
Jentsch, '35	M. undulatus	Amnion, embryos, Testis	42				ZZ	ZW	Second largest

fowl. He stated that in males the unpaired element passes undivided to one pole in the primary spermatocyte division. Later the spermatids which lacked the Z element,² he thought, degenerated. Therefore the male becomes homogametic in accordance with the requirements of genetic experiments. Later work has not confirmed Guyer's observations.

Werner ('27, '31) reported that in ducks and turkeys the female has one more chromosome, the largest element, than the male. According to her scheme two classes of eggs mature, one carrying the Z element, the other a Ww complex; W is the largest element, w a chromosome similar to the Z element.

In the domestic fowl, Hance ('25), Shiwago ('29), Akkeringa ('27), Goldsmith ('28), White ('32), and Popoff ('33) assert that the largest chromosome, a bent element with unequal arms, is unpaired. However, according to Sokolow and Trofimov ('33) and Unger ('36) it is the fifth chromosome which is unpaired in the female. This chromosome is a V-shaped element with arms of equal length; it is perhaps the same chromosome that Suzuki ('30) considered as the sex chromosome. Sokolow, Tiniakow and Trofimov ('36) have summarized their investigations of several of the Gallinaceae. In size and morphology the sex chromosome of these species resembles the fifth element of the fowl complex. Unger ('36) reports that the sex chromosome of fowl, ring necked pheasant, the thrush, *Turdus merula*, and the linnet, *Linota cannabina*, are very similar.

In parakeets Koller (Crew and Lamy, '35) believes that the largest chromosome, and Jentsch ('35) that the second largest chromosome is unpaired in females. In pigeons the largest element is the sex chromosome (Oguma, '27).

A majority of the workers assume that the Z element is not paired with a W chromosome, or leave the question unsettled. However, Shiwago ('24), Akkeringa ('27), and Jentsch ('35) believe that one of the smaller elements has

²In the light of recent investigations it no longer seems desirable to use the symbol XY to represent heterogametic females. Accordingly in our work and in citing other investigations we have used the symbol ZW.

the significance of a W chromosome. Because of the variability in the number of small chromosomes, only by the observation of the maturation divisions could the identity of the W chromosome be established if present. Since the maturation divisions do not occur until the egg is fully formed and ovulated, technical difficulties have prevented observation of the chromosomes during meiosis. Harper ('04) reported sixteen chromosomes in the oogonia and eight in the oocytes of the pigeon. The sex chromosomes were not identified.

An interesting possibility of observing maturation in the female is suggested in the work on ovariectomy in the fowl. Following sinistral ovariectomy, the vestigial right gonad regularly hypertrophies into a testis-like structure. At the present time among 208 ovariectomized fowl examined histologically, twenty-four have shown fertile seminiferous tubules in the right gonad, with germ cells undergoing spermatogenesis. Of these Benoit ('23, '32) found four among thirty-three animals (Zawadowsky ('26) two in ten; Domm ('29, '30) nine in 126, and Padoa ('34) nine in thirty-nine ovariectomized fowl. This paper reports one more case.

None of the previous investigators have considered the chromosome complexes of these exceptional females. Since such material contains female germ cells undergoing spermatogenesis, it can well be expected to reveal the identity of the sex chromosomes.

In birds spermatogenesis has apparently not been studied in its entirety since the early papers of Guyer ('16) and Schöneberg ('13). Hance ('26) remarks on the difficulty of finding mitotic figures in adult testes of the domestic fowl, and Foley ('28) had little success with the English sparrow. In the present study the observations on maturation in the female are supplemented by a study of spermatogenesis in the normal male.

This problem was undertaken at the suggestion of Prof. Emil Witschi. The writer wishes to express his sincere appreciation for helpful advice and criticism given during the course of the investigation.

MATERIAL AND METHODS

To obtain testes showing mitotic figures we have followed the technique suggested by Riley's work on diurnal spermatogenic cycles ('37). He showed that increased daily rations of light or the injection of gonadotropic hormones stimulates testes of sexually inactive sparrows. When the testes of such birds were preserved between midnight and dawn he found mitotic figures in abundance.

A Buff Orpington female was sinistrally ovariectomized 4 days after hatching. Previous to killing at the age of 10 months and 25 days, the bird was injected for 14 days with serum of a pregnant mare. One cubic centimeter was given daily during the first week, and 2 cc. daily the second week. The bird was killed at midnight. The lumbosacral region including the gonads, accessory ducts, and the stump of the cloaca was preserved in Bouin's fixative. A detailed description of this bird is given in a later section. A series of Brown Leghorn females ovariectomized at birth is not included in this paper.

A Brown Leghorn rooster, 11 months old, was killed at 2 A.M. after a 2-week period of injection; 1 cc. (10 R.U.) of pregnant mare serum was given daily. The germ cells in this material are in all stages in maturation.

Each of three Buff Orpington males 33 days old at autopsy had been injected daily for 4 days with 0.2 cc. of pregnant mare serum (0.1 cc. equals 10 R.U.), with 20 mg. of a pyridine extract of bull anterior pituitary (8 mg. equals 1 R.U.), or with 20 mg. of dried turkey anterior pituitary (25 mg. equals 1 R.U.). Sectioned testes show spermatogonial divisions and synizesis stages. The bird injected with turkey pituitary shows fewer divisions.

A fourth male was injected with 20 mg. of dried turkey anterior pituitary daily for 8 days and was killed at 4 A.M. on the following morning at the age of 37 days. Testes show enlarged seminiferous tubules but only spermatogonial divisions.

A fifth male was injected with 20 mg. of the pyridine extract of bull anterior pituitary for 8 days. After an interval of 3 days, 0.2 cc. of pregnant mare serum was given for 10 days and the bird was killed at 2 A.M. The age at autopsy was 50 days. Testes were not greatly stimulated. Sections show gonial divisions and synizesis stages.

The sixth male was injected with 0.2 cc. of pregnant mare serum for 8 days. After an interval of 3 days, six daily injections of 20 mg. of the bull extract were made, and following a second interval of 3 days, 20 mg. of turkey pituitary was given for 3 days and the bird was killed at 2 A.M. The age at autopsy was 52 days. Testes show all stages including a few spermatozoa.

Injections were made approximately 6 hours previous to the hour at which the bird was to be killed at the end of the experiment. During the period of injection the length of the day was increased to 16 hours with 4 hours of artificial illumination.

Tissues were fixed in Bouin's, Allen's B 15, Champy's, and acetic sublimate. Of these Bouin's regularly gave the best results and most of the observations have been made from this material.

After fixation the tissues were dehydrated in a graded series of 35%, 50% and 70% dioxan in water, followed by two changes of pure dioxan. Melted paraffin was added over a period of 2 to 4 hours, the tissue was then transferred to melted paraffin, changed three times, and embedded. Equally good preparations resulted from dehydration following the aniline oil technique suggested by Painter ('22). Sections were cut at 10 μ .

Tissues were stained with Mallory's, with Delafield's hematoxylin counterstained with Congo red, with basic fuchsin by the Feulgen method counterstained with fast green and with Heidenhain's iron hematoxylin. With the last stain the sections were destained in iron alum, or preferably in a saturated solution of picric acid as suggested by Tuan ('30).

Drawings were made with a Spencer camera lucida and a Spencer microscope provided with 1.5 apochromatic objective, aperture 1.3, and a $20\times$ ocular.

DESCRIPTION OF THE OVARIOTOMIZED FEMALE

At the time of autopsy the ovariectomized female had long sickle tail feathers. Neck and saddle hackle feathers were mixed, some of male and some of female type. The comb was 9.8 cm. long and 5.4 cm. high; wattles were 5.1 cm. long. Both were bright red. The bird was not observed to crow.

Examination after autopsy showed at the site of the right gonad a large white, somewhat lobulated, testis-like structure (fig. 1). After preservation it measured 2.5×1.7 cm. Study of the sectioned material shows a testis composed of seminiferous tubules of variable diameter, and cords of germ cells, or single cells separated by connective tissue resembling the tunica albuginea of the normal testis.

Covering the gonad is a thin layer of flat cells taking the place of the ovarian cortex. In localized regions it appears thickened, consisting of cuboidal cells several layers deep. Such patches are connected with the medulla by cords of connective tissue breaking through the tunica albuginea (fig. 35).

Fertile sections of seminiferous tubules contain all stages of germ cell maturation including spermatozoa (fig. 36). For the most part spermatogenesis is entirely similar to that observed in our normal male material, but in some tubules, development evidently takes an atypical course (fig. 37). There are giant cells which in division show large numbers of small chromosomes apparently formed by fragmentation. We have also observed spermatids with pycnotic nuclei.

Sterile tubules (fig. 38) are filled with a fibrous cytoplasm in which no distinct cell boundaries can be seen. The nuclei are located about the periphery of the tubules. The sterile tubules are scattered throughout the gonad but are more numerous in the medial half of the gland.

Running from the region of the right gonad and opening into the cloaca is a prominent wolffian duct (fig. 1). The anterior two-thirds is not coiled but is quite irregular because of annular constrictions. The posterior part is distinctly convoluted until just before it joins the cloaca laterally. At the

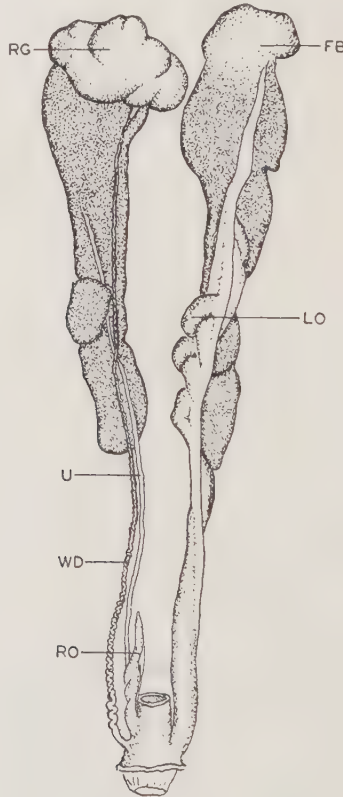


Fig.1 Ovariectomized female, showing right compensatory gonad. LO, left oviduct; RO, right oviduct; WD, wolffian duct; RG, right compensatory testis-like gonad; U, ureter. Camera lucida drawing.

anterior end the duct enlarges and coils about itself in a structure which resembles the epididymis of the male. Cells lining the tubules are columnar and ciliated. A well-developed rete-like formation is visible but there is no open connection with seminiferous tubules. No sperm were found in the wolffian duct.

On the right side is an oviduct 2.4 cm. long (fig. 1). It is a thin-walled structure with a distinct lumen but without ostial or cloacal opening. The left oviduct is 7.5 cm. long and 3 mm. in diameter through the middle convoluted portion (fig. 1).

On the left side at the site of the ovary there is a large smooth body (fig. 1) which on sectioning proved to be fat. No ovarian tissue appears in serial sections, but a small nodule of lymphoid tissue is attached to the epoophoron. The epoophoron is not as extensive as on the right side, although much of it may have been removed in the operation. The left wolffian duct is continuous to the cloaca. Its development is not as extensive as that of the right wolffian duct.

CYTOLOGICAL STUDY OF SPERMATOGENESIS

The histology of the normal avian testis is sufficiently familiar that it need not be described here. Figure 39 illustrates the general appearance of a testis in active mitosis. The course of maturation may be followed from the periphery of a seminiferous tubule, where the spermatogonia are found, to the lumen. About the lumen the nearly mature spermatozoa are arranged in groups attached to Sertoli cells. Spermatogenesis does not proceed in a regular wave, but groups of adjacent cells mature synchronously; primary spermatocyte divisions may occur adjacent to resting spermatogonia, or spermatids beside primary spermatocytes. Several different stages occur simultaneously within a tubule but no one tubule ever shows the complete course of maturation. Some stages are apparently of short duration and are found only after considerable searching.

The course of spermatogenesis has been followed in the adult rooster, in the young stimulated males and in the ovariectomized female. Spermatogonial divisions were difficult to study in the small crowded cells of the adult testis. In the young males, however, the gonidia are large and the chromosomes widely spread. The spermatogonia of the ovariectomized female were almost as large as those in the young stimulated males.

The spermatogonia

There are no significant differences in appearance or behavior of spermatogonia in normal males and in the ovariectomized female. Resting spermatogonia typically show a slightly oval, lightly staining nucleus lying eccentrically in the cell (fig. 2). A fine network is visible upon which appear from place to place small condensations of chromatin. Fine granules of chromatin border the nuclear membrane. There are one to several, but usually two, chromatin-nucleoli. Although these take the usual nuclear stains, they do not appear in



Fig. 2 Resting spermatogonia.

Fig. 3 Spermatogonia shortly before division, showing two central bodies lying in the idiozome.

Fig. 4 Spermatogonial division, side view.

Camera lucida drawings. $\times 2760$.

Feulgen preparations. Between them there is an achromatic nucleolus, larger than the chromatin-nucleoli.

Shortly before spermatogonia divide, there appears a cap of dense cytoplasm, the idiozome of Meves, usually on the side of the nucleus toward the periphery of the seminiferous tubule (fig. 3). Lying in the idiozome is the central body.

Spermatogonial divisions proceed much as do ordinary somatic mitoses. The central body divides into two bodies which migrate to opposite poles of the nucleus. The metaphase spindle is short and broad (fig. 4). In the telophasic reconstruction the central bodies can no longer be seen.

Spermatogonial chromosomes

Spermatogonial metaphase plates (figs. 5 to 8) typically show a number of small chromosomes grouped in the center of the field and surrounded by larger chromosomes at the periphery. Arranged in order as to size, there are six pairs of large chromosomes, a number of medium sized rod-like elements, which are of various lengths, some so short as to be indistinguishable from the small chromosomes lying in the center of the plate.

The large elements show a distinctive morphology. The largest of these is a bent chromosome with submedian spindle fiber attachment. The short arm is a little more than half the length of the long arm. In some figures the first chromosome tapers from heavy ends toward the point of spindle fiber attachment (fig. 6), but often the chromosome has the same diameter throughout its length (figs. 5, 8). The apex is oriented toward the center of the field in metaphase and toward the poles in anaphase.

The chromosomes of the second pair in order of size are likewise J-shaped, with submedian spindle fiber attachment. In this pair the short arm is less than half the length of the long arm. Orientation is the same as in the first chromosome.

The chromosomes of the third pair are straight, tapering from a broad end to a feebly pronounced knob-like enlargement at the other end. At anaphase the small end is directed toward the pole. Spindle fiber attachment is probably terminal. The third chromosome is slightly shorter than the long arm of the first chromosome.

The fourth chromosomes are J-shaped. Spindle fiber attachment is slightly subterminal but so near the end that in many cases the chromosomes appear as rods. In length they are less than the long arm of the second chromosome, or about half the length of the first chromosome.

The chromosome of the fifth order is the only V-shaped element in the entire complex. It is slightly shorter than the chromosomes of the preceding pair, but its morphology is entirely distinctive. Spindle fiber attachment is median. In

male complexes there are two of these elements; in spermatogonia of the ovariectomized female we have never found more than one chromosome of this size and shape. This element then, is the Z chromosome.

The sixth chromosomes are rods which taper slightly. The smaller end is directed toward the center of the metaphase plate. They are shorter than the short arm of the first chromosome but longer than that of the second chromosome.

The seventh pair are rod chromosomes of uniform diameter. They are half as long as the sixth pair. This pair of chromosomes is the first of the rod-like elements of medium size. It and the others of this group differ from the preceding chromosomes in that they show no distinctive morphology.

The material does not permit any conclusive statement as to the number of chromosomes. Spermatogonial figures from both sexes show a broad variation, fifty-one to sixty in the best preparations. The variation, as reported by other investigators, is always in the number of small elements.

Primary spermatocytes

Resting spermatocytes are found against the wall of the tubule or close to it. Their general appearance is similar to that of spermatogonia. There are fewer granules along the nuclear membrane, and the network on which appear the chromatin condensations is finer. The idiozome which surrounds the central body at this time is a thick cap on one side of the nucleus. As maturation proceeds it spreads along the wall of the nucleus to occupy half its circumference. Chromatic and achromatic nucleoli are unchanged in appearance. While

Figs. 5 and 6 Polar views of spermatogonial divisions in the testis-like gonad of the ovariectomized female.

Figs. 7 and 8 Polar views of spermatogonial divisions in the testis of a young Buff Orpington male.

Figs. 9 and 10 Polar views of primary spermatocyte divisions in the testis-like gonad of the ovariectomized female.

Figs. 11 and 12 Polar views of primary spermatocyte divisions in the testis of the Brown Leghorn rooster.

Camera lucida drawings. $\times 1900$.



Figures 5 to 12

still in position on the wall of the seminiferous tubule, the spermatocytes grow to their definitive size (fig. 13). Now begin the characteristic changes in the nucleus. Chromatin nucleoli disappear and the nucleus stains more heavily. The nuclear network increases in complexity, the threads gradually becoming coarser (fig. 14). At the same time the threads begin to draw away from the periphery of the nucleus and form a tangled mass, from which fine chromatin threads project out to the wall of the nucleus (fig. 15). A little later the tangled mass of chromatin threads moves to that side of the nucleus against which lies the idiozome. At this time synapsis occurs. The details of the pairing could not be determined, but when entirely polarized, the strands of chromatin are thick and coarse (fig. 16). During these changes, the achromatic nucleolus is visible between the strands of the fine network, and later when the chromatin has drawn to one side, it may be seen in the clear area of the nucleus.

Now follows a process of unravelling so that the chromatin comes to occupy the entire nuclear space in what appears to be a continuous strand (fig. 17). Occasionally the thread is split, indicating its double nature.

By the frequency with which it is seen, the pachytene stage appears to persist for some time. Eventually, however, the chromatin strands lengthen and become thinner. The nuclear space is now filled with the intricately interwoven thread which in extreme cases approximates a network. Occasionally this thin thread seems to be split. The achromatic nucleolus persists. This stage seems to be a feebly accentuated diffuse stage (fig. 18). During this period the central body divides into two which remain close together.

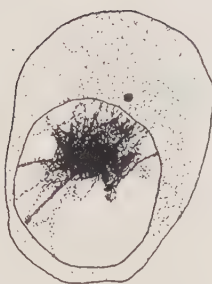
The fine chromatin thread now rapidly contracts. The single thread splits into two, which are twisted about each other in a strepsinema (fig. 19). As contraction continues, the coiled thread breaks up into short lengths which become the tetrads of diakinesis (fig. 20). The bivalent tetrads now coalesce and thicken to form the prophase chromosomes of the first spermatocyte division. The central bodies remain close



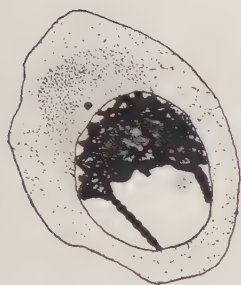
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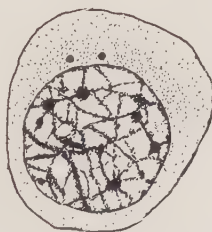
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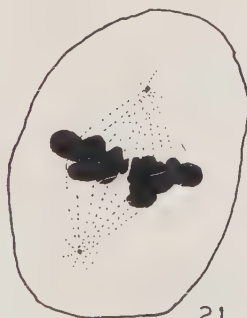
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Fig. 13 Resting spermatocyte.

Fig. 14 Early spireme.

Fig. 15 Synaptene stage.

Fig. 16 Bouquet stage.

Fig. 17 Pachytene stage.

Fig. 18 Diffuse stage.

Fig. 19 Strepsinema breaking up into tetrads.

Fig. 20 Diakinesis.

Fig. 21 Primary spermatocyte division normal male, side view.

Camera lucida drawings. $\times 2760$.

together until just before division and then rapidly separate. The metaphase spindle is narrow, and the central bodies relatively farther apart than in gonial divisions (fig. 21). During these changes the heterochromosome is not distinguishable by pycnosis or any peculiarities of behavior.

Spermatocyte chromosomes

In figures 9, 10 and 40 are shown the first spermatocyte chromosomes of the female, and in figures 11 and 12 those of the male. The resemblance between the spermatocyte and spermatogonial chromosomes is obvious. The bivalent spermatocyte tetrads are much heavier and for that reason the finer structural details, such as the knob-like enlargement of the third spermatogonial chromosome, are not apparent.

The fifth chromosome requires special comment. In male spermatocytes it is a thick tetrad only slightly bent. On the contrary in the metaphase plates of female material, the fifth chromosome closely resembles the fifth spermatogonial element. It is slender, whereas the other chromosomes are thick, indicating that these are the result of conjugation of homologous gonial elements. Since the fifth chromosome is unpaired in the female, it retains its gonial appearance.

In the female, the behavior of the fifth chromosome during the first spermatocyte division is significant. This chromosome passes undivided to one pole of the spindle. It has never been seen to divide. Furthermore it is not paired on the equatorial plate with any of the smaller chromosomes. It may regularly be seen migrating to one pole of the spindle while the rest of the field remains in the metaphase condition (figs. 22, 23, 41). In extreme cases it has moved to a position between the central body and the cell wall while the autosomes are just beginning their anaphase movement (fig. 24). In male spermatocytes the fifth tetrad segregates in the usual manner.

The primary spermatocyte chromosomes in the ovariotoximized female were especially favorable for counting. Good preparations in which the small elements are well stained

regularly show forty chromosomes. Occasionally thirty-eight or thirty-nine are found. In these cases it is probable that one or two of the small elements are concealed by the large ones. In male spermatocytes, where for some reason the chromosomes were not as well spread as in the female, counts gave accordingly smaller numbers.

Secondary spermatocytes

Resting secondary spermatocytes are slightly smaller than spermatogonia. The nucleus shows several scattered clumps of chromatin (fig. 25). The central body lies in the idiozome.



Figs. 22 to 24 Side views of primary spermatocyte divisions in the ovariectomized female; shows characteristic appearance of the fifth chromosome as it passes undivided to one pole of the spindle ahead of the autosomes. Camera lucida drawings. $\times 2500$.

The resting stage is of short duration, followed by the secondary spermatocyte division. Chromosomes in the metaphase plate are closely crowded. Five to seven large chromosomes and several smaller elements may be seen in the better preparations (fig. 26). The spindle is long and narrow (fig. 27). There is no evidence of a second pairing as reported by Guyer ('16).

Spermiogenesis

The nucleus of a resting spermatid shows one to three chromatin spheres and a scattering of granules along the nuclear membrane. A small achromatic nucleolus is present. The central body lies in a small indistinct idiozome (fig. 28).

The first nuclear change in spermiogenesis is a spinning out of the chromatin spheres into a network and an accumulation of chromatin along the nuclear membrane (fig. 30). The network of chromatin material then begins to contract laterally. At first there is only a slight elongation in the anterior-posterior direction, which steadily increases as contraction diminishes. At this time the sperm head is seen lying in a clear space surrounded by a membrane (fig. 31). Shortly after contraction ceases the sperm head becomes attached to a Sertoli cell. The head grows enormously long and tapers to a fine point. The chromatin network becomes more and more compact and eventually the sperm head stains homogeneously (fig. 32). At about the time the head has achieved its maximum length, the membrane becomes closely applied to the sperm head which shortens (fig. 33). Shortly afterward, spermatozoa are released into the lumen of the seminiferous tubule.

Only a few of the details in the formation of the tail could be observed. In the resting spermatid a central body lies in a small idiozome. Later as the chromatin spins out into a network, the central body elongates into a rod which will eventually form the tail filament (figs. 28, 29). At first the rod lies tangentially to the nucleus but later turns and moves toward it until one end touches the nuclear membrane. A little later the end appears to have entered the nucleus (fig. 30). The rod increases in length, the posterior end passing out of the cell. As it elongates, it becomes thinner and stains less and less intensely so that in later stages it may be seen only with great difficulty. At the time when the membrane enclosing the sperm head appears there is seen a fine thread

Fig. 25 Resting secondary spermatocyte.

Fig. 26 Secondary spermatocyte division, polar view.

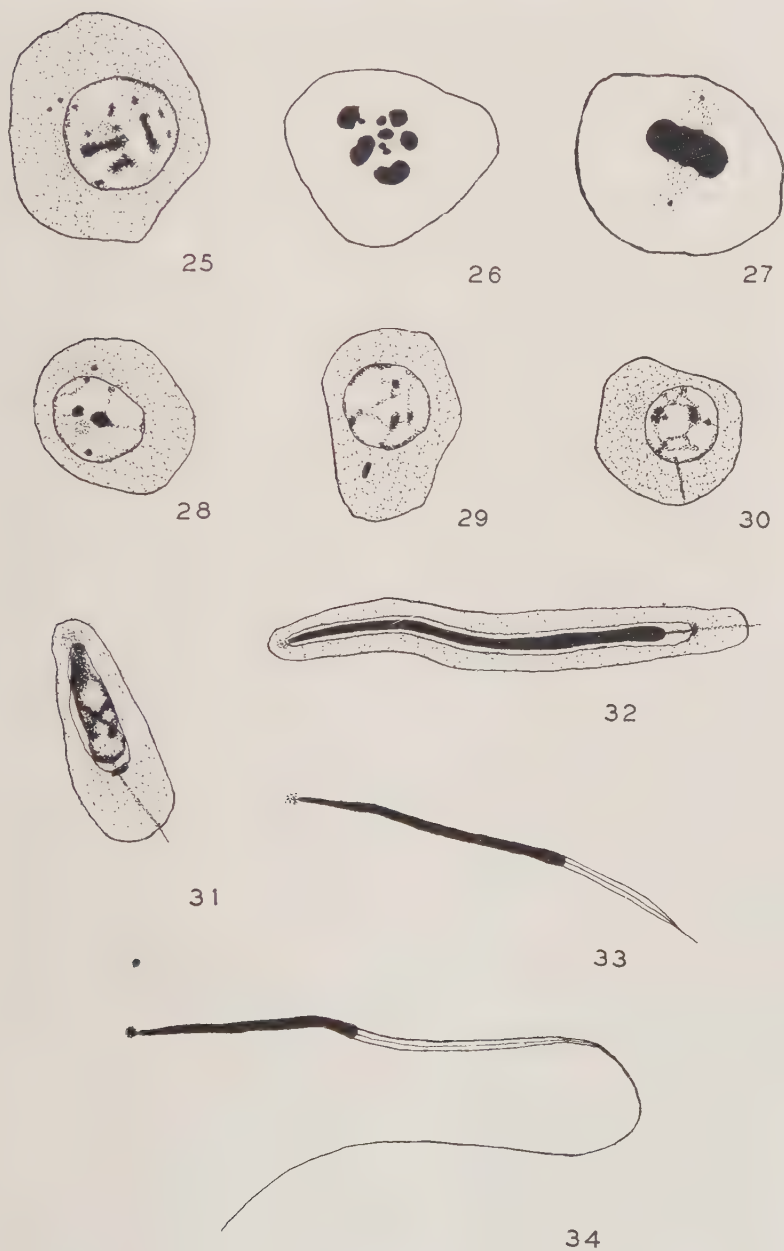
Fig. 27 Same, equatorial view.

Fig. 28 Resting spermatid.

Figs. 29 to 33 Several stages in the transformation of the spermatid into the spermatozoon. (In figs. 28 and 29 only part of the tail filament is represented.)

Fig. 34 Spermatozoon free in lumen of a seminiferous tubule.

Camera lucida drawings. $\times 2760$.



Figures 25 to 34

which leads away from the sperm head, and passing through the enclosing membrane appears to be continuous with the tail filament (fig. 31). At this point on the membrane there is a small mass of dark-staining material whose origin could not be determined (fig. 31). Later this material is no longer visible. About the time the membrane flattens against the sperm head, the tail becomes encased in a sheath (fig. 33).

The acrosome is formed from idiozome material which migrates around the nucleus to the anterior end before elongation begins (fig. 30). Spermatozoa in the seminiferous tubules show acrosome, head and tail (fig. 34).

DISCUSSION

Breeding experiments on the inheritance of sex-linked characters in birds prove that sex determination is of the ZZ-ZO or ZZ-ZW type. Until recently the cytological evidence for this conclusion has not been satisfactory. In fowl both the largest and the fifth largest chromosomes have been designated as the sex chromosome and both conclusions have been repeatedly corroborated. It is significant that those who believe that the first chromosome is unpaired in the female have not reported a consistent individual morphology for the largest elements. If the pairing of the chromosomes is based upon the determination of their length, the well recognized difficulty of accurate measurement may account for what now appears to be an erroneous conclusion.

Sokolow and Trofimov ('33) have described and their figures show a consistent individual morphology for the seven largest chromosomes. With this important advance the sex chromosome was identified as the fifth largest element in the complex.

While the investigations of the Russian workers is admirable one must still admit that in material as difficult as avian chromosomes, there remains the danger of individual interpretation in making up series of paired chromosomes. For a complete case, observation of the appearance and behavior of the heterochromosome during maturation divisions is highly

desirable. Obviously only such evidence could prove the presence or absence of a W chromosome. In species with the more common male heterogamety this would be a simple matter, but in avian material the technical difficulties of observation are almost insurmountable in the normal female. Fortunately in fowl there exists the unusual opportunity in the potentiality for male differentiation of the vestigial right gonad of the female.

Witschi ('32) has shown in amphibians that the cortex and medulla of the gonad are sources of substances inducing respectively male and female differentiation of the germ cells. In addition, cortical inductor inhibits male differentiation, while medullary inductor suppresses female differentiation. In fowl Witschi ('35) has concluded that there is a "primary hereditarily fixed deficiency in the right cortical inductor." This deficiency is manifested in the infrequency with which the germ cells are attracted to the cortex at the time the germ cells are distributed during embryonic development. As a result the right gonad is primarily a medullary structure with occasionally a cortical rudiment. It is clear that the presence of the left ovary is instrumental in suppressing development of the right gonad, since upon ovariectomy the right gonad hypertrophies into a testis-like structure. This compensatory growth of the right gonad recalls the reverse result obtained in the experiments of Harms ('23) and Ponse ('24) on Bidder's organ in toads. In all cases following extirpation of the testis, Bidder's organ (a cortical rudiment) hypertrophies into a functional ovary in the male.

In the fowl any germ cells present in the right gonad at the time of sinistral ovariectomy then undergo male differentiation. Brode ('28) believes that germ cells persist in the right gonad up to 3 weeks after hatching and then degenerate. However, Padoa ('34) reports that right gonads showing spermatogenesis occurred with equal frequency in birds ovariectomized between 3 and 6 months of age and in those operated between 18 and 23 days after hatching.

Save for the presence of a well-developed cortex, the left ovary is essentially similar to the right gonad. If the cortex is surgically removed, the medulla is able to respond with testicular development. Benoit ('23), Zawadowsky ('26 b), Domm ('27), and Padoa ('34) have noted in ovariectomized fowl that there is a development of the remaining medulla into a sterile testis.

We have shown that these germ cells which are differentiating into spermatozoa still retain the female chromosome complex. Obviously differentiation in the male line is not accompanied by any change in the female chromosome pattern. Such evidence proves that differentiation of the germ cells in these cases is controlled by the somatic medulla and not directly by their chromosomal makeup.

Similar considerations have been derived by Witschi ('29) from his genetical analysis of adult hermaphrodites of the grass frog, *Rana temporaria*. He artificially fertilized eggs from hermaphrodites with normal sperm, and normal eggs with sperm of the hermaphrodites. The sex ratio of the progeny demonstrated that both eggs and sperm of the hermaphrodite were genetically female. Crew ('21) also gave proof that the testes of adult hermaphrodites were genetically female. On cytological examination Witschi ('24) reports a diploid number of twenty-six in spermatogonia and oogonia of the hermaphrodites and a haploid number of thirteen. Stohler ('28) also found that Bidder's organs and the functional gonads of both sexes have the same chromosome complexes.

As yet no genetical analysis of the ovariectomized females has been made. Domm ('30) artificially inseminated several normal females with motile sperm obtained from the right gonad of ovariectomized fowl. All the eggs laid were infertile. His technique was successful when sperm of normal males was used. Domm suggests as an explanation for the negative results that since the sperm were taken from the gonad they may not have been physiologically mature. Other workers have reported that spermatogenesis is atypical in the right

gonad and that the spermatozoa are not normal. However, Zawadowsky and Zubina ('26) have a drawing of spermatozoa in the seminiferous tubules of their hen Vega which appear normal. In our material some of the spermatozoa are structurally quite normal.

It may be concluded that sex reversal in ovariectomized fowl, as in adult hermaphrodite frogs, does not involve a change in genetic constitution.

SUMMARY AND CONCLUSIONS

1. A Buff Orpington female sinistrally ovariectomized 4 days after hatching developed a testis-like structure on the right side. The bird was stimulated with gonadotropic hormones and was killed during the night, when mitotic divisions are most apt to occur in the testis. The appearance and behavior of the chromosomes during maturation are compared in this ovariectomized female and in testes from similarly stimulated males.

2. A study of spermatogenesis in normal males and in the ovariectomized female is reported.

3. The form of the large chromosomes is described in spermatogonial and spermatocyte divisions. The fifth chromosome in order of size, a V-shaped element with median spindle fiber attachment, is the sex chromosome. In male spermatogonia the element is paired; in female spermatogonia it is single.

4. The fifth spermatocyte tetrad in males is a heavy element formed by the pairing of homologous spermatogonial chromosomes. In the female the corresponding element is a slender chromosome resembling the fifth spermatogonial chromosome.

5. The fifth spermatocyte chromosome in the female passes without dividing to one pole of the spindle ahead of the other chromosomes. In males the fifth tetrad segregates, one chromosome moving to each pole.

6. The exact number of chromosomes could not be determined. Spermatogonial counts vary between fifty-one and sixty. Spermatocyte chromosomes are more easily counted.

In good preparations the number is between thirty-eight and forty. The number forty is found most frequently and probably represents the true monoploid chromosome number.

LITERATURE CITED

- AKKERINGA, L. J. 1927 Die Chromosomen bei einigen Huhnerrassen. *Z. mikr. anat. Forsch.*, Bd. 8, S. 324-341.
- BENOIT, J. 1923 a Transformation expérimentale du sexe par ovariectomie précoce chez la poule domestique. *C. R. Acad. Sci.*, T. 177, pp. 1074-1077.
- 1923 b Sur la structure histologique d'un organe de nature testiculaire développée spontanément chez une poule ovariectomisée. *C. R. Acad. Sci.*, T. 177, pp. 1243-1246.
- 1923 c A propos du changement expérimental de sexe par ovariectomie chez la poule. *C. R. Soc. Biol.*, T. 89, pp. 1326-1328.
- 1932 Étude physiologique, histologique et histophysiologique de l'inversion sexuelle de la poule déterminée par l'ablation de l'ovaire gauche. *Arch. Zool. Exp. Gen.*, Bd. 73, S. 1-112.
- BORING, ALICE M. 1923 Notes on chromosomes of the domestic chicken. *Science*, vol. 58, p. 73.
- BORING, A. M., AND R. PEARL 1914 The odd chromosome in the spermatogenesis of the domestic chicken. *J. Exp. Zool.*, vol. 16, pp. 53-84.
- BRODE, MALCOLM 1928 The significance of the asymmetry of the ovaries of the fowl. *J. Morph.*, vol. 46, pp. 1-57.
- CREW, F. A. E. 1921 Sex-reversal in frogs and toads. A review of the recorded cases of abnormality of the reproductive system and an account of a breeding experiment. *J. Gen.*, vol. 11, p. 141.
- 1923 Review on Punnett's Heredity in Poultry. *Nature*, vol. 112.
- CREW, F. A. E., AND R. LAMY 1935 Autosomal colour mosaics in the Budgerigar. *J. Gen.*, vol. 30, pp. 233-242.
- CUTLER, D. W. 1918 On the sterility of hybrids between the pheasant and the Gold Campine fowl. *J. Gen.*, vol. 7, pp. 155-166.
- DOMM, L. V. 1927 New experiments on ovariectomy and the problem of sex inversion in the fowl. *J. Exp. Zool.*, vol. 48, pp. 31-173.
- 1929 Spermatogenesis following early ovariectomy in the Brown Leghorn fowl. *Arch. Entwmech. Org.*, Bd. 119, S. 171-187.
- 1930 Artificial insemination with motile sperm from ovariectomized fowl. *Proc. Soc. Exp. Biol. and Med.*, vol. 28, pp. 316-318.
- FOLEY, J. O. 1928 A note on the spermatogenetic wave in the testes of the adult English sparrow (*Passer domesticus*). *Anat. Rec.*, vol. 41, pp. 367-371.
- GOLDSMITH, J. B. 1928 The history of the germ cells in the domestic fowl. *J. Morph.*, vol. 46, pp. 275-315.
- GUYER, M. F. 1902 Spermatogenesis of normal and hybrid pigeons. *Univ. of Cincinnati Bull.* 22, vol. 3, pp. 1-61.
- 1909 a The spermatogenesis of the domestic guinea (*Numida meleagris dom.*). *Anat. Anz.*, vol. 34, pp. 502-513.

- GUYER, M. F. 1909 b The spermatogenesis of the domestic chicken (*Gallus gallus* dom.). *Anat. Anz.*, vol. 34, pp. 573-580.
- 1916 Studies on the chromosomes of the common fowl as seen in testes and in embryos. *Biol. Bull.*, vol. 31, pp. 221-268.
- HANCE, R. T. 1924 The somatic chromosomes of the chick and their possible sex relations. *Science*, vol. 59, pp. 424-425.
- 1926 a A comparison of mitosis in chick tissue-cultures and in sectioned embryos. *Biol. Bull.*, vol. 50, pp. 155-159.
- 1926 b The chromosomes of the chick soma. *Biol. Bull.*, vol. 51, pp. 443-449.
- 1926 c Sex and the chromosomes of the domestic fowl. *J. Morph.*, vol. 43, pp. 119-145.
- HARMS, W. 1921 Umwandlung des Bidderschen Organs in ein Ovarium beim Männchen von *Bufo vulgaris* Laur. *Zool. Anz.*, Bd. 53, S. 253-265.
- HARPER, E. H. 1904 The fertilization and early development of the pigeon's egg. *Am. J. Anat.*, vol. 3, pp. 349-386.
- HELM, URSULA 1934 Über die gesetzmässige Lage der Gemini im Kernraum. *Z. Zellf. mikr. Anat.*, Bd. 21, S. 791-806.
- JENTSCH, SEYFRIED 1935 Die Chromosomen des Wellensittichs (*Melopsittacus undulatus* Sh.) untersucht in somatischen Geweben und in der Spermatogenese, nebst einigen Bemerkungen über Kernfragmentation im Amnion. *Z. Zellf. mikr. Anat.*, Bd. 23, S. 607-626.
- KEMP, TAGE 1930 Über die somatischen Mitosen bei Menschen und warmblütigen Tieren unter normalen und pathologischen Verhältnissen. *Z. Zellf. mikr. Anat.*, Bd. 11, S. 429-444.
- LECAILLON, A. 1910 La variation du nombre des chromosomes dans la segmentation de l'oeuf non fécondé de la poule. *C. R. Soc. Biol.*, T. 69, pp. 34-36.
- LOYEZ, MARIE 1906 Recherches sur le développement ovarien des oeufs méroblastiques à vitellus nutritif abondant. *Arch. d'Anat. micros.*, T. 8, pp. 69-397.
- OGUMA, K. 1927 Studies on the sauropsid chromosomes. I. The sexual difference of chromosomes in the pigeon. *J. Coll. Agric. Sapporo*, vol. 16, pp. 203-227.
- PADOA, E. 1934 Ricerche sperimentale sulla sessualità nei polli. Estratto dall'*Archiva di Anat.*, T. 33, pp. 242-420.
- PONSE, K. 1924 L'organe de Bidder et le déterminisme des caracteres sexuels secondaires du crapaud (*Bufo vulgaris*). *Rev. suisse de Zool.*, T. 31, pp. 177-220.
- POPOFF, W. W. 1933 Über die Zahl der Chromosomen und über die Heterochromosomen des Haushuhns. *Z. Zellf. mikr. Anat.*, Bd. 17, S. 341-346.
- RILEY, G. M. 1937 Experimental studies on spermatogenesis in the house sparrow, *Passer domesticus* (Linnaeus). *Anat. Rec.*, vol. 67, pp. 327-351.
- SAGUCHI, S. 1931 Über das Verhalten des Nucleolus bei der Mitose in Kulturgewebe nebst Bemerkungen über die Chromosomenzahl beim Huhn. *Ref. Ber. Biol.*, Bd. 16, Cytologischen Studien, H. 3.

- SCHÖNEBERG, K. 1913 Die Samenbildung bei den Enten. Arch. mikr. Anat., Bd. 83, S. 324-369.
- SHIWAGO, P. J. 1924 The chromosome complexes in the somatic cells of male and female of the domestic chicken. Science, vol. 60, pp. 45-46.
- 1929 Über den Chromosomenkomplex der Truthennen. Z. Zellf. mikr. Anat., Bd. 9, S. 106-115.
- SMITH, GEOFFREY 1912 Studies in the experimental analysis of sex. 9. On spermatogenesis and the formation of giant spermatozoa in hybrid pigeons. Quart. J. Micr. Sci., vol. 58, pp. 159-170.
- SOKOLOV, N. N., G. G. TINIAKOW AND J. E. TROFIMOV 1934 On the chromosomes of the domestic turkey (Russian). Biol. Z., Bd. 3, S. 648-654.
- 1936 On the morphology of the chromosomes in Gallinaceae. Cytologia, vol. 7, pp. 466-489.
- SOKOLOV, N. N., AND J. E. TROFIMOV 1933 Individualität der Chromosomen und Geschlechtsbestimmung beim Haushuhn (Gallus dom.). Z. indukt. Abstamm. u. Vererb-Lehre, Bd. 65, S. 327-352.
- SONNENBRODT, H. 1908 Die Wachstumsperiode der Oocyte des Huhns. Arch. mikr. Anat., Bd. 72, S. 415-480.
- STOHLER, R. 1928 Cytologische Untersuchungen an der Keimdrüsen mittel-europäischer Kroeten. Z. Zellf. mikr. Anat., Bd. 7, S. 400-475.
- SUZUKI 1930 Zool. Mag. Tokyo, vol. 42, p. 358.
- TINIAKOW, G. G. 1934 Pfauhahn- und Hennebastarde und eine vergleichende Analyse der Karyotypen ihrer Elteren. Biol. Z. (russ.), Bd. 3, S. 41-63.
- TROFIMOV, J. E., AND G. G. TINIAKOW 1933 Der Karyotyp von Phasianus colchicus verglichen mit dem von Gallus domesticus. Biol. Z. (russ.), Bd. 2, S. 33-43.
- TUAN, HSU-CHUAN 1930 Picric acid as a destaining agent for iron alum hematoxylin. Stain Technology, vol. 5, pp. 135-138.
- UNGER, HELENE 1936 Beitrag zur Chromosomenforschung der Vögel. Z. Zellf. mikr. Anat., Bd. 25, S. 476-500.
- WERNER, O. S. 1927 The chromosomes of Indian Runner Duck. Biol. Bull., vol. 52, pp. 330-372.
- 1931 The chromosomes of the domestic turkey. Biol. Bull., vol. 61, pp. 157-164.
- WHITE, M. J. D. 1932 The chromosomes of the domestic chicken. J. Gen., vol. 26, pp. 345-350.
- WITSCHI, E. 1924 Die Entwicklung der Keimzellen der Rana temporaria. I. Urkeimzellen und Spermatogenese. Z. Zellen-u. Gewebelehre, Bd. 1, S. 523-561.
- 1929 Studies on sex differentiation and sex determination in amphibians. III. Rudimentary hermaphroditism and Y chromosome in Rana temporaria. J. Exp. Zool., vol. 54, pp. 157-223.
- 1934 Genes and inductors of sex differentiation in amphibians. Biol. Rev., vol. 9, pp. 460-488.
- 1935 Origin of asymmetry in the reproductive system of birds. Am. J. Anat., vol. 56, pp. 119-141.

- ZAWADOWSKY, M. M. 1926 a Materiale zur Analyse des Gynandromorphismus. I. Kastration der Finken und Gimpel. Arch. Entw. Mech. Org., Bd. 108, S. 563-571.
- 1926 b Bisexual nature of the hen and experimental hermaphroditism in hens. Trans. Lab. Exp. Biol. Zoopark Moscow, vol. 2, pp. 164-179.
- ZAWADOWSKY, M. M., AND E. ZUBINA 1929 Hahnenfederige Fasanenweibchen im Lichte der Embryogenese der Geschlechtsdrüsen. Arch. Entw. Mech. Org., Bd. 115, S. 52-92.

PLATE 1

EXPLANATION OF FIGURES

- 35 Thickened cortex in the right gonad of the ovariectomized female.
- 36 Section of seminiferous tubule of the right gonad, showing spermatozoa.
- 37 Section of seminiferous tubule in the right gonad showing several primary spermatocyte divisions and degenerated cells in the center of the tubule.
Photomicrograph. $\times 210$.

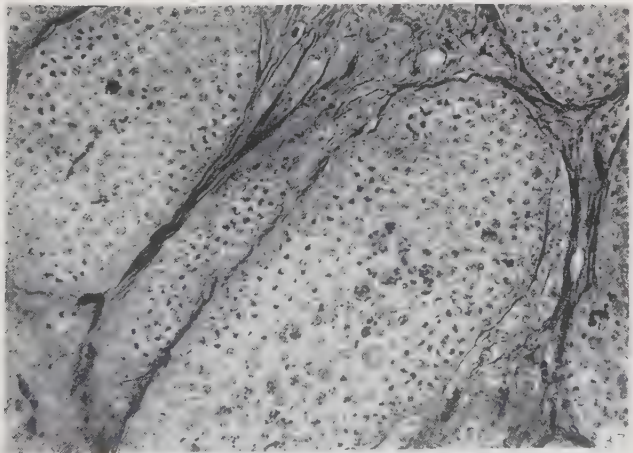
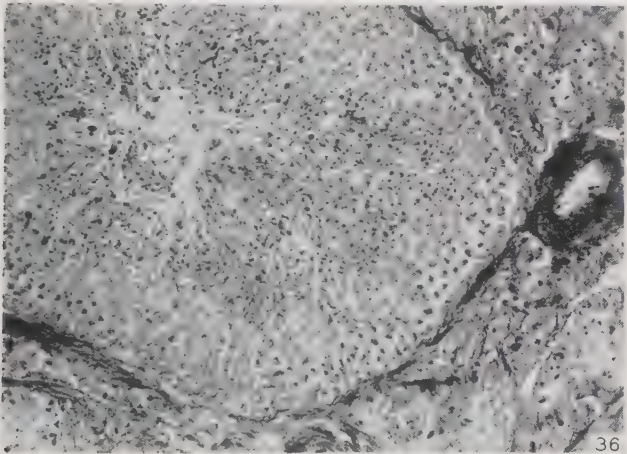
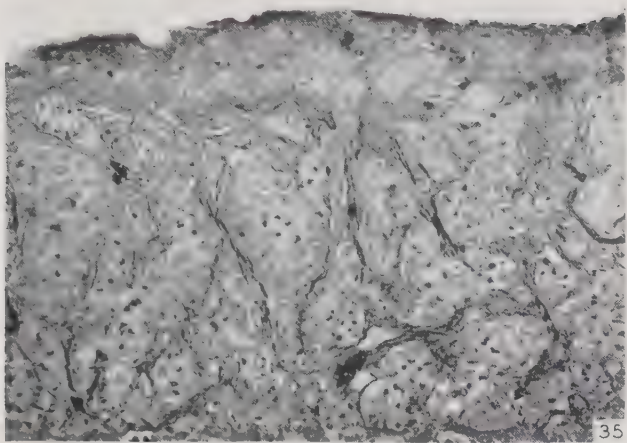


PLATE 2

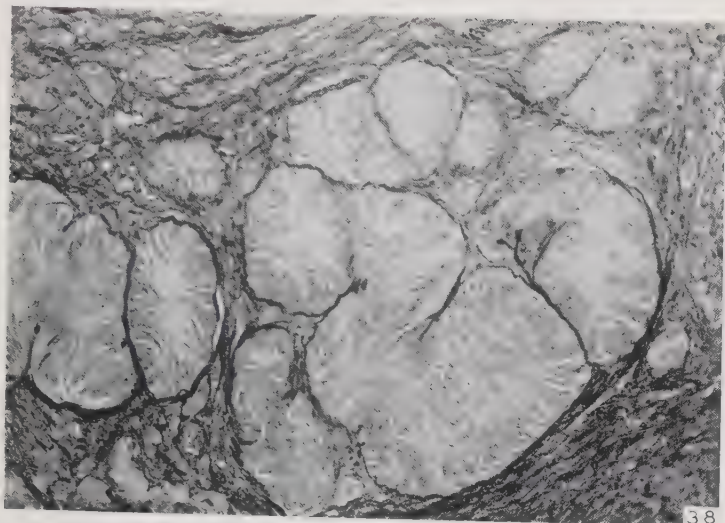
EXPLANATION OF FIGURES

38 Sterile seminiferous tubules in the right gonad of the ovariectomized female. Photomicrograph. $\times 130$.

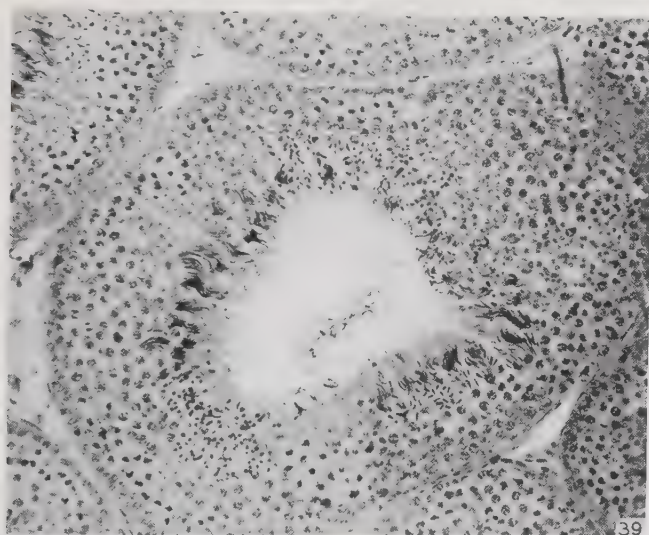
39 Seminiferous tubule of the Brown Leghorn rooster, showing many of the stages in maturation. Photomicrograph. $\times 210$.

40 Photomicrograph of a primary spermatocyte division from the ovariectomized female. The same cell is represented in figure 9. $\times 1930$.

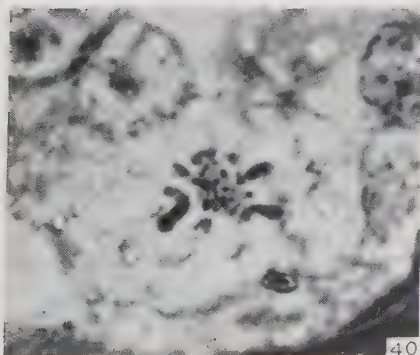
41 Side view of a primary spermatocyte division in the ovariectomized female. The same cell is represented in figure 22. Photomicrograph. $\times 1500$.



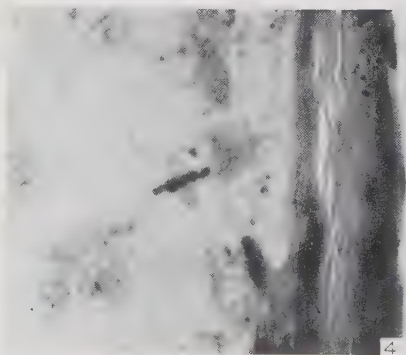
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39



40



41

PLATE 3

EXPLANATION OF FIGURES

We are indebted to Mr. Max Poser of the Bausch and Lomb Optical Co. for the photographs on this plate. They were not received until after the engravings had been made and for this reason figures 40 and 44 are duplicates.

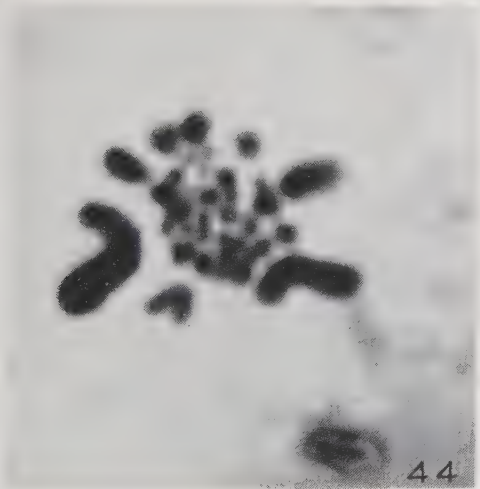
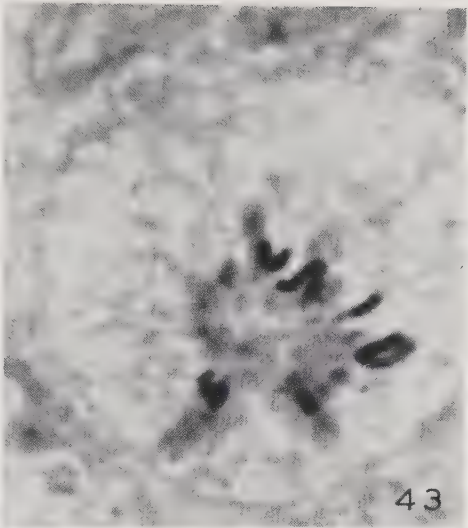
42 Polar view of a spermatogonial division in the testis-like gonad of the ovariectomized female. The same cell is represented in figure 6.

43 Polar view of a spermatogonial division in the testis of a young Buff Orpington male. Represented in figure 8.

44 Polar view of a primary spermatocyte division in the ovariectomized female. Represented in figure 9.

45 Side view of a primary spermatocyte division in the ovariectomized female. Represented in figure 24.

Photomicrographs, $\times 4500$.



TWO CASES OF PERSISTENT LEFT SUPERIOR VENA CAVA IN MAN

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THREE FIGURES

The two cases of left superior vena cava illustrated here were observed in adult male subjects. They make an interesting addition to a well-known group of venous anomalies described by Marshall (1850) and others.

The first case (figs. 1 and 2) occurred in a man, J.P., no. 841, age 84 years, white, who died October 16, 1934. His height was 5 feet, 5 inches, weight 95 pounds. He was thin in build and well preserved. The diagnosis and cause of death was given as heart disease.

The heart was large and displaced to the right. There was a left-sided pericarditis, moderate in extent, with some fibrous adhesions of the pericardial layers. The oblique pericardial sinus was completely obliterated by this adhesion, but the pericardial sac could be readily stripped from the posterior surface of the heart. Over the remainder of the cardiac surface the pericardial sac was free from the heart. The surrounding lymph glands were very large and definitely pathologic. It is probable that the pericarditis accounted in part for the displacement of the heart to the right.

The arch of the aorta (2) was somewhat enlarged and elongated. The descending thoracic aorta was diseased, bent and elongated as a result of inflammatory involvement of local lymphatics.

Beneath the mediastinal glands and beneath the remnant of the thymus and running across the front of the arch of the

aorta there was a slender and irregularly branching plexus of veins (24) which connected the two superior venae cavae. The small tributaries to this venous rete came from the individual lymph glands, from the upper anterior border of the pericardial sac, and from the thymus gland. This plexus of veins probably represented the venous communication which in the normal course of development gives origin to the transverse portion of the left innominate vein. The left superior vena cava (26) passed down almost straight crossing the left side of the aortic arch. Before entering the pericardial sac it received the vein formed by the left accessory hemiazygos and the superior intercostal vein (30). After entering the pericardial sac the left vena cava (33) passed down in front of the root of the left lung. Turning to the right it was continued into the enlarged coronary sinus (22) which opened into the right atrium, as seen in figure 2.

The occurrence of a left vena cava superior in this case is evidently due to a persistence of the left common cardinal vein of the early embryo.

MEANING OF NUMBERS ON ALL FIGURES

1, aorta ascendens	21, plexus cardiacus superficialis
2, arcus aortae	22, sinus coronarius
3, arteria anonyma	23, trachea
4, arteria carotis communis	24, vena anonyma communicans
5, arteria mammaria interna	25, vena anonyma dextra
6, arteria pulmonis	26, vena anonyma sinistra
7, arteria pulmonis sinistra	27, vena cava inferior
8, arteria subclavia sinistra	28, vena cava superior
9, arteria thymica	29, vena cordis magna
10, atrium dextrum	30, vena intercostalis suprema sinistra
11, atria sinistrum	31, vena jugularis interna
12, cartilago cricoidea	32, vena mammaria interna
13, cartilago thyroidea	33, vena obliqua atrii sinistri
14, cor	34, vena pericardiacophrenica
15, costa prima	35, vena pulmonis sinistra
16, ductus arteriosus	37, vena subclavia
17, ductus thoracicus	38, vena thyroidea ima
18, glandula thyroidea	39, venae thymicae
19, nervus vagus	40, vena vertebralis
20, pericardium	

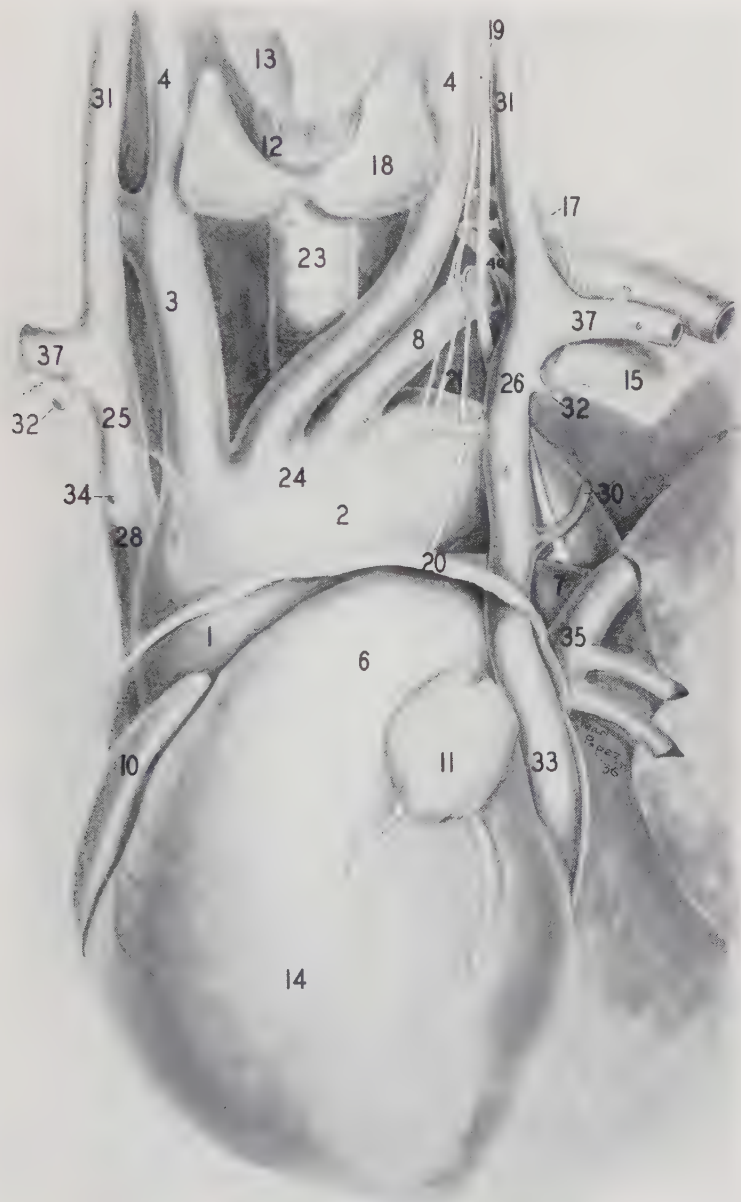


Fig. 1 Persistent left vena cava in the first case, viewed from the front and from the left side.

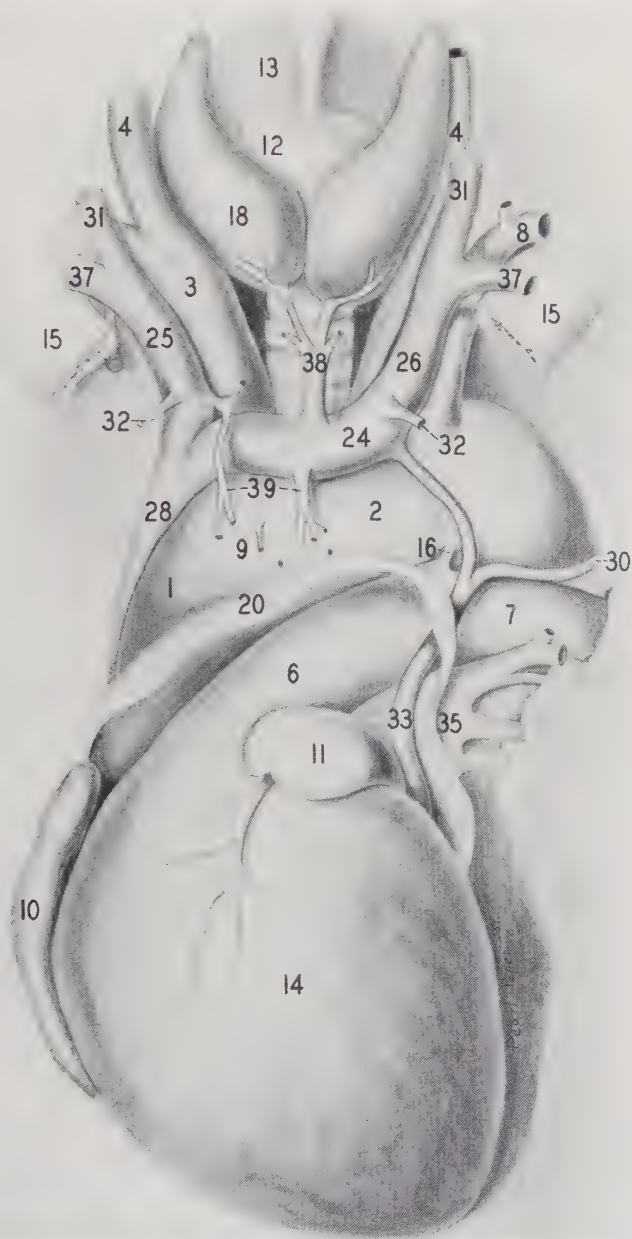


Fig. 3 Partial persistence of the left superior vena cava in the form of a large oblique vein of the left atrium.

between the aortic arch and the left pulmonary artery, it received the left superior intercostal vein (30).

There is no doubt from the position of this vein (33) that its intrapericardial portion represents a persistence of the left common cardinal vein of the embryo.

DISCUSSION

These two cases of persistent left superior vena cava were the only two observed in about 635 cadavers dissected in this laboratory. Such cases have, in general, been interpreted as a fortuitous persistence of primitive venous connections in the neck region: connections which usually undergo a number of transformations to reach the conditions found in the adult.

The embryonic history of the cardinal veins is well known. Dorsal to the heart the anterior cardinal and posterior cardinal veins unite to form the common cardinal veins on each side. Both common cardinals enter the sinus venosus. Normally the terminal portion of the left common cardinal persists as the coronary sinus; sometimes a more distal portion remains as the oblique vein of the heart. The terminal portion of the left posterior cardinal vein persists as the left superior intercostal vein. During the eighth week of embryonic life the anterior cardinals begin to communicate by a transverse venous anastomosis through the thymic region at the upper level of the aortic arch. This communication enlarges and becomes the terminal segment of the left innominate vein. Its connection with the left anterior cardinal becomes attenuated, but it may persist as a small vessel, as in case 2, or as a fibrous cord, or it may be entirely lost.

In the first case the small communicating vein (24) was clearly related to the thymus gland and the surrounding structures. Observations made by Szawlowski (1891) on human embryos and by Anikiew ('09) on the guinea pig have led them to the conclusion that the veins draining the thymus gland are concerned in the formation of the anastomosis which becomes enlarged to form the terminal portion of the left innominate vein. It will be noted that in the second case, in

addition to the thymic veins (39), the thyreoid ima vein (38) was also a prominent tributary of the transverse portion of the left innominate vein (24).

The comparative anatomy and the development of the great anterior veins which enter the heart is well known from the work of Marshall (1850) and others.

McCotter ('16) collected from the literature sixty-four cases similar to case 1, only seventeen of which could be definitely determined as having been observed in adults. To this may be added cases described by Huffmire and Bower ('19), Keyes and Keyes ('25), Hurley and Coates ('26-'27), Odgers ('27-'28) and Thompson ('29), and some additional cases which these men cite from other authors.

More remarkable are the cases of a partial or total absence of the right superior vena cava. Smith ('16) reported one and found that thirteen such cases had been previously recorded. More recently Halpert ('27) has reported another case. Some other vascular anomalies in this region are discussed by Harris, Gray and Whitney ('27) who attempt a more extensive interpretation of these fortuitous changes in blood vessels connecting with the heart.

SUMMARY

Two cases of persistence of the left common cardinal vein as a superior vena cava in adult human subjects are described and illustrated. These vessels empty into the coronary sinus.

In one case the right and left superior venae cavae are of equal size. There is no direct communication between the two. Only an irregular plexus coming from the thymus and surrounding structures connects them.

In the other case the persistent left superior vena cava is small and its terminal portion corresponds in position to the oblique vein of the left atrium (the oblique vein of Marshall). The main part of the left innominate vein is large and communicates in the usual way with the right innominate.

The cases are interpreted as a fortuitous persistence of the left common cardinal system of the embryo.

LITERATURE CITED

- ANIKIEW, A. 1909 Zur Frage über die Entwicklung der Vena anonyma sinistra. Anat. Anz., Bd. 34, S. 24-29.
- HALPERT, B. 1927 Complete situs inversus of the vena cava superior. Anat. Rec., vol. 35, p. 38.
- HARRIS, H. A., S. H. GRAY AND C. WHITNEY 1927 The heart of a child aged 22 months presenting an anomalous vein, etc. Anat. Rec., vol. 36, pp. 31-49.
- HUFFMIRE, A. P., AND G. C. BOWER 1919 A case of persistence of the left superior vena cava in an aged adult. Anat. Rec., vol. 17, pp. 127-129.
- HURLEY, L. E., AND A. E. COATES 1927 A case of right-sided aortic arch and persistent left superior vena cava. J. Anat., vol. 61, pp. 333-339.
- KEYES, D. C., AND H. C. KEYES 1925 A case of persistent left superior vena cava with reversed azygos system. Anat. Rec., vol. 31, pp. 23-26.
- MARSHALL, J. 1850 On the development of the great anterior veins in man and mammals; etc. Phil. Trans. Royal Soc., vol. 140, pp. 133-170.
- MCCOTTER, R. E. 1916 Three cases of persistence of the left superior vena cava. Anat. Rec., vol. 10, pp. 371-383.
- ODGERS, P. N. B. 1928 A case of bilateral superior venae cavae in the adult. J. Anat., vol. 62, pp. 221-223.
- SMITH, W. C. 1916 A case of a left superior vena cava without a corresponding vessel on the right side. Anat. Rec., vol. 11, pp. 191-198.
- SZAWLOWSKI, I. 1891 Zur Morphologie der Venen der oberen Extremität und des Halses. Dissert. St. Petersburg (und Ergebn. d. Anat. u. Entwickl. v. Merkel u. Bonnet, Bd. 3, 1893, Weisbaden 1894, S. 380-382, Stiedas Referat).
- THOMPSON, I. M. 1929 Anatomical note. Venae cavae superiores dextra et sinistra of equal size in an adult. J. Anat., vol. 63, pp. 496-497.

THE DEVELOPMENT OF RAT EMBRYOS IN A CIRCULATING MEDIUM

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TWO FIGURES

Previous experiments by Nicholas and Rudnick ('34), Goss ('35) and Jolly and Lieure ('36) have shown that rat embryos possess the capacity for limited development during certain critical stages. All these investigations have employed some variation of the plasma clot method—that of Jolly and Lieure, a non-clotting but viscous medium.

Wherever plasma, embryonic extracts, serum and other animal fluids are used as tissue culture media, it has long been recognized that there is a series of variables which prevent reliable repetition of the experiment. Plasma obtained from the same animal on successive days may give entirely different results. The same plasma stored in the ice box may undergo changes which are sufficient to affect growth of similar biological materials, even when used within 24 hours of the initial bleeding. Complex formulae (Baker, '36) which take into account the various things which can and have been used in the study of the growth of tissues have been evolved, but even these pseudosynthetic media cause reactions at variance with the normal.

So much attention has been paid to the media necessary for tissue proliferation—hitherto the sole criterion for its efficacy—that little effort has been expended upon interpretation of the physiologic readjustments that occur in the tissues. If growth is not obtained, various hypothetical entities are called into being to explain the negation of result, such as species

specificity, special hormones or even a bios. Whatever the factors may be, it is clear that when fluids of insufficiently known composition are used, we have been exceedingly fortunate to be able to repeat the experiment. Originally invented by Harrison ('07) for the study of the specific problem of nerve outgrowth—to the solution of which it supplied the crucial experiment—the method has been used in many ways. Recently, in a somewhat modified form, it has been applied by Holtfreter ('31) and by Oppenheimer (unpublished data) to problems of amphibian and teleost development. In both instances, the fluids used contained nothing but inorganic constituents so that for continuance of development of the embryonic fragments, reliance was placed upon the cell inclusions. Until the reactions of the specific cells to the medium in which they are cultured are known, the answers to many problems must be fragmentary. It is even possible that the cells of one germ layer may be stimulated to more rapid development than those of another even in an inorganic environment.

The problems of development are complicated by an additional criterion which does not occur in normal tissue culture work, viz., the cells of which the embryo is composed must proliferate; further they must differentiate and must organize, not only into fragmentary parts but into a complete and working whole which can outgrow the simple morphological boundaries of its differentiating units and develop into a combination of morphological entities which will possess the capacity for interlocking with the functional complexities that will arise later. Not only must this type of interlocking development occur, but what is most important, it must take place in a synchronous relation with the varying conditions arising both inside and outside the embryo.

So far there has been little attempt to advance beyond the type of culture which was first developed by Harrison ('09). Brachet's experiments with the developing rabbit ('13) have formed a beginning and while his studies have been carried to a greater degree of completeness, the amount of growth and differentiation which he secured has rarely been excelled.

The only attempts to amplify the results in such a way as to make them applicable to the general problems of development have been those of Lewis ('29). He has photographed various mammalian eggs in the living state and has advanced our knowledge of the reacting capacity of the embryonic materials to an astonishing degree.

The idea of using circulating fluids for the nourishment of the tissues is not a new one and really goes back to the original perfusion experiments conducted by the physiologists during the middle of the last century. The experiments performed in the Rockefeller Institute with the aid of Lindbergh's machine ('35), have shown conclusively that we are able to attack the problem of the survival of tissues from new and unexpected angles. With an increase in the accuracy of the method of attack, much increase in our knowledge of this problem can be expected.

In conversation with Colonel Lindbergh, it seemed that a type of apparatus simpler than that necessary for the study of adult organs would be requisite. At the same time it was realized that the conditions necessary for development are quite different from those for maintenance and those requisite for the proliferation of cells from already formed tissues. A combination of these factors together with the capacity for observation under the microscope led to the design of the instrument which is here given. The chamber is a modified Carrel flask (fig. 1) similar to that used by botanists in studying plant tissues with changes of the fluid medium. To this was added a small arm which connects with the air system or with a filter system depending upon whether the compressed air line is the source of the operating circulation (compare fig. 2) or whether the small circulator is attached to a water pump which sucks the air through a filter; the end result is the same in each case, since the air bubble lifts a small column of the fluid and carries it through the arm to the reservoir where the air escapes. The amount of circulation can be regulated readily, and also, in the case of the use of compressed air, the pressure under which the fluid is kept is regulated by use of suitable pinch cocks on the rubber tube above

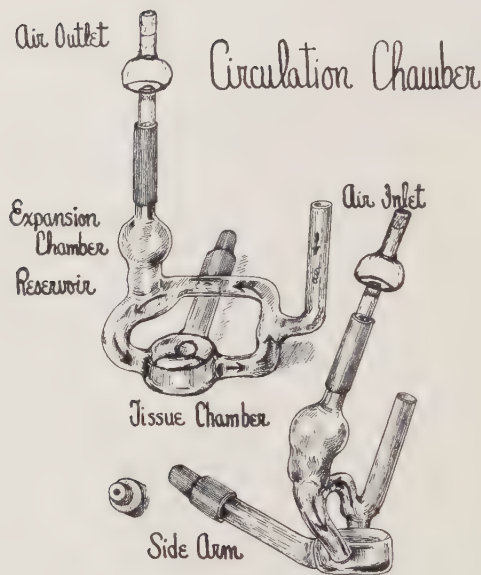


Fig. 1 For description see text.

Fig. 2 An incubator with a battery of circulating chambers. Compressed air (20 to 25 pounds, pressure) enters the system at the gauge on the right of the figure, passes through rubber pressure tubing to a glass tube containing a cotton filter at the angle above the wash bottle. The wash bottle (10 liter capacity) is filled three-quarters full with sterile water. The air bubbles from the end of the glass tube and is both washed and moisture saturated. It then passes through a second sterile cotton filter and through a rubber tube to the incubator chamber where it enters a manifold in which is located a third cotton filter. Five rubber tubes lead away from each manifold and connect with the circulating chambers, a pinch cock control (shown on left of figures) affording the regulation of the speed of circulation. In the pressure experiments the pinch cock was placed on the rubber tube just below the air outlet shown in detail in figure 1.

The circulating chambers are fastened to a brass bar by means of metal clips and each unit of five circulators can be removed from the incubator chamber for observation under the binocular dissecting microscope. If desired each individual chamber may be placed on the stage of a compound microscope without disturbing its circulation. Since the chambers are but 12 to 14 mm. in depth, the embryo can be observed in detail. The two bulbs indicated in the posterior part of the incubator serve as heater elements under control of a deKhotinsky thermostat.

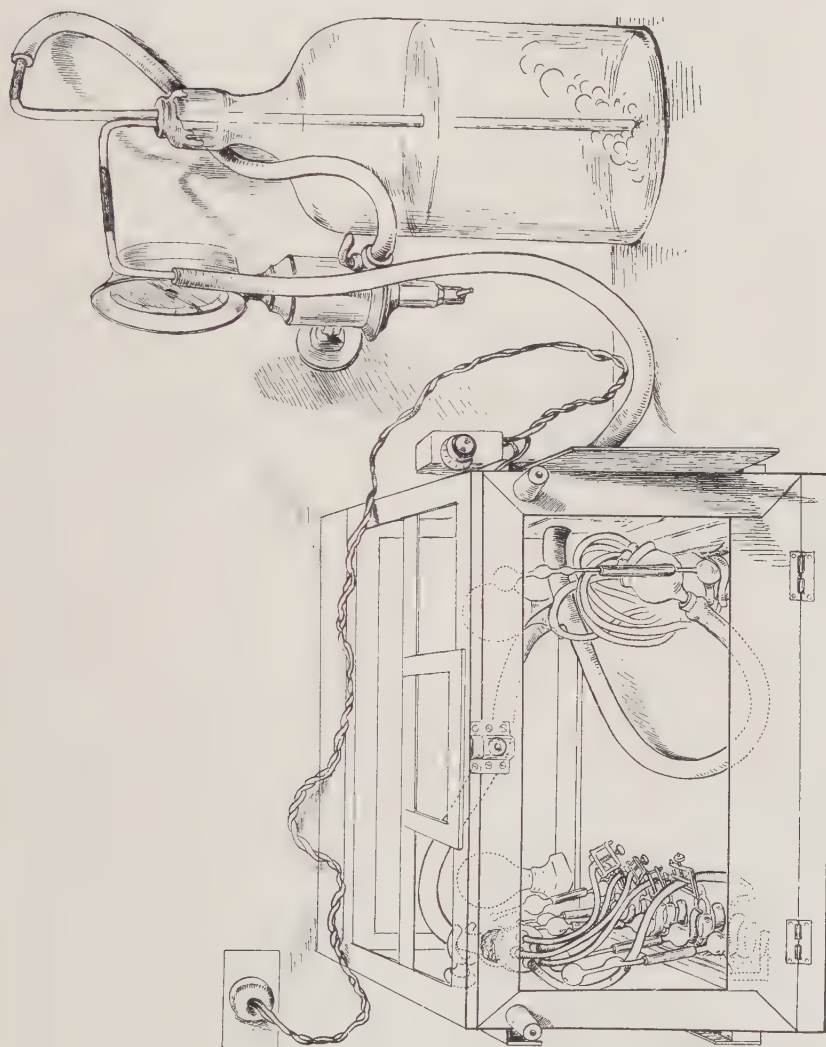


Figure 2

the expansion chamber. The pressure may be measured by means of a small manometer. The entire apparatus is simple, easy to sterilize and to clean. It requires the minimum of fluid—a necessity when working with either rat or mouse plasma or with concentrations of other costly chemicals. As at present constructed, a single circulator contains about 2 cc. of fluid which is sufficient to support development under normal conditions for 96 hours. Without opening the chamber, extra fluid can be added merely by injecting it through the rubber stopper that closes the end of the side arm (fig. 1). The entire circulator can be placed either under the binocular dissecting microscope or under the low power of the compound microscope for observation. Altogether the circulator is ideal for embryological work.¹ Furthermore, since a circulator keeps conditions within the culture homogeneous, at least it should test the long debated question as to whether a tissue-culture medium changes its chemical properties or whether its materials are exhausted by the tissues being cultivated in it.

EXPERIMENTAL DATA

Since the beginning of the year, more than 1000 rat embryos of various stages have been subjected to experiment in the circulators described above. The latter are combined in a battery of 10 units which are placed in an incubator controlled by a Cenco-deKhotinsky thermo-regulator (fig. 2). Since it had already been found that a certain limited growth in a plasma clot with homologous plasma and embryonic extract could be obtained from rat embryos in these stages in which there is either a proliferation of the mesoderm or an initial differentiation of organs (compare Nicholas and Rudnick, '38) such stages were cultured first. Also they will be described first, since these experiments are essentially a test of the apparatus rather than a test of the embryo.

¹ All the glass parts for this circulator are made of Pyrex glass blown by Mr. John Bee of the Macalaster Bicknell Company, of New Haven. I wish to thank Mr. Bee for his cooperation which has made this work possible.

Determination of the proportions of fresh rat plasma and embryonic extract that would give the greatest amount of growth in rat embryos was based on previous experience. While this was by no means ideal, it proved a convenient starting point. As the method for the preparation of such culture media will soon be available (Nicholas and Rudnick, '38), it will not be repeated here. It should be stated, however, that since the heparinized plasma of the rat clots quickly and a fluid medium is required, the material was either frozen—in which case it seldom clotted—or was diluted with Ringer's solution, tyrode, Pannett-Compton, 0.8% NaCl, or some biological fluid. This was done in full recognition of the fact that we are still unaware of the exact nature of the fluids necessary for growth and development.

Employing fluids of the same nature as those used previously, it was found that the pH changed rather rapidly in the circulators since the plasma alone did not possess sufficient buffer action for long continued exposure to the conditions imposed. It was also found that the addition of Tyrode's solution in the proportions recommended by Parker ('37), furnished a buffer action which was sufficient to maintain the fluids at a fairly constant pH over a period of 48 to 96 hours. Since the most rapid changes in the embryo occur within this period, and differentiation is complete in non-circulating cultures at about this time, this seemed to offer a suitable time limit during which the embryos could be adequately exposed in order to determine the efficacy of the circulating medium. The following fluids were employed, using the early 9-day rat as the test animal.

- a, rat plasma (heparinized)
- b, rat plasma plus 0.8% NaCl
- c, rat plasma plus Tyrode
- d, rat plasma plus Pannett-Compton
- e, rat serum in all the above combinations
- f, rat plasma plus hemoglobin in various concentrations up to 4% by weight.
- g, rat plasma plus Tyrode, inactivated by heating at 55°F. for 1 hour
- h, guinea pig plasma (in combination as above)
- i, cat plasma (alone and in combination)
- j, digests of hen plasma, fibrin and liver

All of these media gave good results occasionally, but their absolute reliability was not predictable. In some cases the embryos would show just as much initial development in Tyrode alone as in the combinations of fluids that were being tested. Since all the fluids contained everything that could possibly be required for the normal growth and development of an embryo, without consistently permitting either, the attempt was made to find some of the constants involved in these reactions. The problem was attacked by using a fluid that had given good reactions with 100% of the test animals over the first 24-hour period. The method of preparation was carefully checked, particularly the sterilization methods. Since the plasma was untreated except for the addition of heparin (1 unit, Connaught per cubic centimeter)—1 cc. of heparin to 7 cc. total blood—the preparation of the Tyrode or the added fluid was the only thing to be considered. It was found that sterilization by autocleaving in some way changed some of the constants of the Tyrode so that it was no longer stable under the conditions of the experiment. This was true whether there was an embryo present in the circulator or not. Apparently one or more of the constituents failed to act as buffers after autoclaving when placed under the conditions of constant circulation. Thereafter and in the last forty experiments, the Tyrode was sterilized by using a Berkefeld filter. This in no way impaired the buffer action, was quicker, and avoided all chance of that precipitation which sometimes occurs unaccountably in autoclaved material. Our routine now calls for Berkefeld filtration wherever possible.

All cultures, whatever the combinations of fluids, had added to them a small quantity of 0.04% phenol red which enabled one to see at a glance whether the reactions within the medium were occurring rapidly and also toward which side, acid or alkaline. While it is recognized that this is not an adequate indicator in solutions containing protein, it reveals much that is going on in the medium, especially the existence of bacteria or moulds.

In order to test whether the circulation of the medium had any effect upon the embryos, one-half of the circulators were used for still cultures. In these cases, irregularities appeared more frequently and development was less regular both in quantity and quality than in the five that were in the circulating medium. All cultures became alkaline during the first 24 hours, the still cultures changed more than the circulating ones. There was a greater tendency for clotting in the plasma of the still cultures and in every way these seemed to parallel the conditions found in depression slide cultures or in watch glass cultures. Embryos carried in comparable circulating fluids increased in growth perceptibly over those in the still cultures. Their differentiation was likewise increased and disturbances similar to those noted in the still cultures appeared much later, if at all, in the circulating cultures.

In order to control the pH of the circulating media, gases (CO_2 and O_2) were used as the circulating agents. These were attached to the compressed air inlet and bubbled through the sterile water contained in the large carboy shown in figure 2. While there was a pronounced effect, as would be expected when the gases were used individually or in combination, the media did not react to the change as readily nor was the effect upon the embryo nearly so marked as expected. In one experiment where carbon dioxide was used throughout the entire 48-hour run, the embryos showed very little effect although the media changed very perceptibly. Similarly where oxygen was used in excess of the normal, little result in stimulating or accelerating development was obtained. The indirect effect upon the pH of the medium constitutes the sole reason for the use of these gases. It is concluded that in the earlier stages of rat development, during which the tissues of the primary germ layers are developing into their definitive parts, there is little stimulation of direct cellular action by these gases.

The findings in respect to temperature proved to be quite similar. Varying the temperature within rather wide limits seemed to have slight effect upon comparable stages of rat embryos. In these experiments, gradations of 5° ranging

from 70° F. to 110° F. were employed. While slight differences were found to occur, development seemed remarkably constant for the given stage at each of the various levels tried. The temperature range during which a rat embryo may exist is much wider than one would suppose. When older stages, after the initiation of circulation are used, there is a marked cessation of the heart beat as the temperature falls below 80° F. The heart beat can be restored to normal when the temperature is again raised above this point. The heart begins to beat, however, almost as well in the lower as in the higher temperatures. Having ascertained these facts, the temperatures in the majority of the experiments were kept close to the normal mammalian temperature.

As the work proceeded—especially as a result of the experiments with the gases—it became increasingly evident that a more comprehensive study of the hydrogen ion concentration would have to be undertaken.² As would be expected, the plasma relations of the media used were found to have an initial reaction slightly alkaline, ranging from pH 7.2 to 7.8. It had been found by experiment that in most cases, development was most rapid and regular when the initial pH of the Tyrode which was added to the plasma was slightly below pH 7.0, i.e., from pH 6.5 to 6.8, but in all cases the pH rose slightly during the first 24 hours. The embryos themselves at the given stage seemed to be able to accommodate themselves to an astonishing range in pH providing the change is not too rapid. Embryos have been grown successfully at pH's ranging from 5.8 to 8.5. This shows the range of accommodation of the embryo but tells nothing about the conditions under which it normally develops. Further experiments along this line will be performed in the future.

The combination of the facts determined above have led to the utilization of a third component of the media in which the embryos are now being cultured and one which seems to be the most successful yet tried. A mixture of either rat or cat

² The purchase of a Beckman pH meter was made possible through the Elizabeth Thompson Fund and an accurate determination by the electrometric method was carried out.

plasma, pig amniotic fluid and Tyrode in approximately equal amounts seems to provide a fluid in which the greatest approach to normal growth and development can be realized. This combination is the most stable of any of those studied so far, its initial pH (which is slightly below neutrality as determined electrometrically) being maintained over long periods of time.

The amniotic fluid of the pig will not by itself promote growth and development. When, however, Tyrode's solution or plasma is added, growth and differentiation can take place, more of each resulting from a combination of the two substances than occurs in either alone. Nothing could be much more heterologous than the combination of pig amniotic fluid, cat plasma, and Tyrode's solution, yet in such a wierd combination a rat embryo will grow and differentiate. At present it is impossible to state whether the finer details of this process are identical with those occurring in normal development; the gross aspects and the main features of the microscopic picture fall well within the limits of normal variability.

The effect of pressure upon the fluids seems to play little or no part in the developmental set up. When the suction system is used for generating circulation within the chamber, the fluid and its contained embryos are under a slightly negative pressure. The effects of this on growth and development are no different from the positive secured with a direct pressure of 1 kg.

In the main, the experiments here reported are confined to one stage of development and the end of the reported observations is given as the 48-hour period. The experiments, however, have been carried considerably further and normal development can be carried through 96 hours and sometimes longer. At the end of this period, the substances within the fluid media are not exhausted, as can be shown by using the same media upon embryos of the same stage as the cultured embryos at the beginning of the experiment. Ninety-six hours after the beginning of culture, however, the vessels in the yolk sac epithelium become gorged with blood and circulation ceases. This and other puzzling circumstances remain for future study.

SUMMARY

From the above experiments several things are clear. A rat embryo, of the stage used, grows and develops better in a circulating medium than it does in a 'still' culture. In heterologous plasma mixtures, it possesses the capacity to regulate itself to variations in temperature, pressure and hydrogen ion concentration. It is evident that early growth, as indicated by the mass enlargement of the embryo and the distension of the yolk sac epithelium, can occur under quite varying conditions. Differentiation, as shown by the organs and systems formed, can proceed with uniform rate and regularity. The present system of handling enables the investigator to observe directly the results of manipulation and to control within limits the varying changes to which the embryo is subjected. The experimental approach has now reached the state at which the reaction of the embryo to small units, either biological or chemical, can be studied in detail.

LITERATURE CITED

- BAKER, L. E. 1936 Artificial media for the cultivation of fibroblasts, epithelial cells and monocytes. *Science*, vol. 83, p. 605.
- BRACHET, A. 1913 Développement 'in vitro' de jeunes vésicules blastodermique de lapin. *Arch. d. Biol.*, T. 28, pp. 448-503.
- GOSS, C. M. 1935 Double hearts produced experimentally in rat embryos. *J. Exp. Zool.*, vol. 72, pp. 33-49.
- JOLLY, J., AND C. LIEURE 1936 Sur les cultures des orufs de Mammifères. *C. R. Soc. Biol. Paris*, T. 122, pp. 723-726.
- HARRISON, R. G. 1907 Observations on the living developing nerve fiber. *Proc. Soc. Exp. Biol. and Med.*, vol. 4, p. 140.
- HOLTFRETER, J. 1931 Ueber die Aufzucht isolierter Teile des Amphibienkeimes. II. Züchtung von Keimen und Keimteilen in Salzlösung. *Roux' Arch. f. Entw. d. Org.*, Bd. 124, S. 404-466.
- LEWIS, W. H., AND P. W. GREGORY 1929 Cinematographs of living developing rabbit eggs. *Science*, vol. 69, pp. 226-229.
- LINDBERGH, C. A. 1935 An apparatus for the culture of whole organs. *J. Exp. Med.*, vol. 62, pp. 409-431.
- NICHOLAS, J. S., AND D. RUDNICK 1934 The development of rat embryos in tissue culture. *Proc. Nat. Acad. Sci.*, vol. 20, pp. 656-658.
- 1938 Development of rat embryos of egg-cylinder to head-fold stages in plasma cultures. *J. Exp. Zool.* (In press.)
- PARKER, R. C. 1937 Studies on the production of antibodies in vitro. *Anat. Rec.*, vol. 67, p. 38.

THE ANATOMY OF THE INGUINAL AND HYPO- GASTRIC REGIONS OF THE ABDOMINAL WALL ¹

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TWO TEXT FIGURES AND THREE PLATES (SIX FIGURES)

The writers have frequently witnessed the misdirected efforts of students to convert inguinal and hypogastric anatomy into a complex arrangement of layers, bands and falces which would conform to the descriptions and figures which their textbooks contain. Impressed, the while, with the fundamental simplicity in stratification presented by these musculo-aponeurotic layers, the authors undertook to study and describe them anew. The first portion of the investigation will be reported in the present article. It is hoped that the several reports will be of service to dissector and to surgeon.

The descriptions herein presented are based upon permanent records obtained from the dissection of 125 consecutive specimens, or 250 inguino-hypogastric regions. The illustrations represent the anatomy of five selected cases; two of the five (specimens A and D) display the common type of strata, the other three (specimens B, C and E) types less frequently encountered.

SPECIMEN NO.	RACE	AGE, YEARS	HEIGHT, INCHES	WEIGHT, POUNDS	FIGURE NO.
A	Negro	50	68	160	3, 5, 6
B	White	60	69	130	7, 8
C	Negro	40	67	140	4
D	White	50	68	180	1
E	White	58	68	190	2

¹ Contribution no. 242 from the Anatomical Laboratory of Northwestern University Medical School.

EXTERNAL OBLIQUE MUSCLE

In the area with which we are at present concerned, the external oblique muscle presents no anatomical features which are essentially at variance with those attributed to it by the most accurate of modern textbook descriptions. One feature is of sufficient importance to warrant emphasis here, namely the extent to which the aponeurosis of the external oblique may be separated medially from that of the internal oblique next below. The point of fusion of the two aponeuroses does not follow a vertical line, nor does it coincide with the line formed by the lateral border of the rectus muscle (fig. 3); it is oblique and often semilunar, though situated considerably medial to the linea semilunaris. In 125 cadavers, while the average distance from the line of fusion to the median line of the body, at the level of the anterior superior iliac spine was 4.1 cm., just above the pubic crest this distance was but 1.1 cm. The aponeurosis of the external oblique muscle therefore contributes very little to the hypogastric portion of the rectus sheath.

The aponeurosis of the external oblique muscle is usually described as possessing an auxiliary insertion inferiorly, additive to the attachments represented by the inguinal ligament and the crura of the subcutaneous inguinal ring; this insertion, the reflected inguinal ligament, is rarely present; in well-developed form it occurred bilaterally in only one of 125 specimens examined, and poorly developed, unilaterally, in three others.

INTERNAL OBLIQUE MUSCLE

The fascicles of the lower third of the internal oblique muscle, as they leave the iliac spine, follow either a transverse (fig. 3) or an oblique course (fig. 7); their direction is always oblique near the inguinal ligament. Near the linea semilunaris the muscle fibers terminate, giving place to an aponeurotic plate which, over the hypogastric area, constitutes the tendon of insertion of the muscle. The relation of muscle to aponeurosis in most instances is approximately in the ratio

of two to one, the lateral two-thirds being made up of muscle, the medial one-third of aponeurosis. Usually in the male the lowermost fibers of the internal oblique curve downward in their course, pass over the spermatic cord, and attach to the tubercle and pecten of the pubis; they are specially named aponeurotic inguinal falx (conjoined tendon). However, even in those instances in which the portion of the internal oblique adjacent to the inguinal ligament is completely muscular (figs. 3 and 7), the arching character of the inguinal 'falx' is certainly not an arresting feature although described and figured by most authors. The margin becomes falciform only when the fibers of the cremaster layer are sharply retracted from it; then a small triangular cleft becomes apparent (fig. 3 at arrow); the muscle fibers forming the superior boundary of this cleft are the only discernible representation of the aponeurotic inguinal 'falx.'² It therefore seems reasonable to regard the falx as an artifact, evident only after the muscle fibers have been separated into two groups by the cleft, and after the external layer of the investing fascia has been removed. This supposedly 'falciform' portion of the internal oblique has long received attention, drawing it from other more important features, such as the relation of the layer to the rectus muscle; thus while it is regularly recognized that the aponeurosis of the internal oblique passes in front of the rectus muscle, the extent to which the latter is overlapped by the former may not be so well known. Not only the aponeurosis of the internal oblique, but also a medial strip of its muscular portion overlies the rectus muscle (see dotted line, figs. 3 and 5).

² There is even a difference of opinion as to what constitutes the 'falx.' Most authors consider it a conjoined tendon, a fusion of the muscles: Testut ('21) on the other hand does not even consider the falx inguinalis and the conjoined tendon as the same structure, the latter, in his opinion, lying superficial to the former. What Testut labels falx inguinalis we consider only a slip from the under surface of the transversalis fascia (aponeurosis of the transversus abdominis muscle) which passes medially to invest the rectus muscle in the lower portion of the abdomen.

The above description applies to the arrangement of the internal oblique in 84% of the 250 inguinal regions studied—those in which the aponeurotic portion of the internal oblique is limited to its medial part (as in fig. 3). In the remaining 16%, the layer is less muscular than it is in specimen A (fig. 3). So aponeurotic are some of the internal oblique layers, that, since the transversus abdominis is often likewise so, much of the inguinal part of the anterior abdominal wall is composed of three superimposed aponeurotic plates (fig. 2). Thus in specimen B the aponeurosis of the internal oblique is more extensive than it is in specimen A, having a maximum width of 5.5 cm. (fig. 7; compare fig. 8), the corresponding width usually being about 1.5 cm. (fig. 3); the aponeurosis extended lateralward to within 2 cm. of the anterior superior iliac spine. In specimen E (fig. 2) the aponeurosis was similar to that of the preceding specimen in that it extended lateralward as a fibrous stripe, but different therefrom in that the extension lay just above the cord, not opposite the iliac spine.

TRANSVERSUS ABDOMINIS MUSCLE

Contrary to conventional descriptions, the transversus abdominis muscle definitely does not terminate caudally in an arch composed of muscle fibers; by the same token, it does not form a roof for the inguinal canal, but is carried outward, from the abdominal inguinal ring, as a thin contribution to the layers of the spermatic cord. No muscle fibers are exchanged between the transversus and internal oblique muscles; descriptions of such fusions are doubtless based upon a failure to recognize the accessory internal oblique muscle, described by Chouke ('35), which frequently intervenes between the internal oblique proper and the transversus abdominis.³

The lunular interval which is almost invariably described and figured as separating the inferior border of the muscle fibers from the inguinal ligament and labeled 'transversalis

³ Chouke states that this muscle is present in 82% of the cases whereas we found it present in 40% (30% and 50% in two series of thirty-five bodies each).

fascia' is not an interval in the true sense, but merely an area of the transversus layer in which muscle fibers are replaced by an aponeurosis; the latter, we find, is in no way different from the medially placed thick aponeurosis of insertion. Both the medial and the inferior portions of the aponeurosis are invariably complete (fig. 8). However, the latter portion, in occasional specimens is seemingly deficient just medial to the abdominal inguinal ring, as in our specimen C (fig. 4). In this case (representing 1% of the bodies studied) the aponeurosis at first appeared to be deficient, but by bringing traction on the cord, the very thin medial portion of the aponeurosis was demonstrated.

The extent to which the inguinal portion of the transversus may be free of muscle fibers is shown by the following data (226 inguinal regions) on the maximal perpendicular distance between the muscle fibers and the inguinal ligament: 0 mm., four specimens; 1 to 10 mm., twenty-eight specimens; 11 to 20 mm., fifty-eight specimens; 21 to 30 mm., seventy-nine specimens; 31 to 40 mm., thirty-seven specimens; 41 to 50 mm., seventeen specimens; 51 to 55 mm., three specimens.

TRANSVERSALIS FASCIA

The aponeurosis of insertion of the transversus muscle may occupy almost the entire inguinal region (specimens E and B, figs. 2 and 8) or merely form a vertical stripe situated lateral to the rectus muscle (specimen A, fig. 5). Whether extensive or scanty, its relation to the rectus muscle is clear: its main bulk passes anterior to the muscle in that portion of the hypogastric region lying inferior to the semicircular line, being joined in succession by the aponeuroses of external, and of internal oblique muscles (fig. 1). Near the point where the muscle fibers of the transversus terminate, the two layers of investing fascia of the muscle fuse indivisibly with the aponeurosis (fig. 1, at arrow *b*), so that the three become a single layer. The outer layer of investing fascia has never been designated by a special name; the inner one, which is the stronger of the two, has long been termed the 'transversalis

fascia' (fig. 1, at arrow *a*). As seen in specimen A, the latter lies on the deep or peritoneal surface of the transversus muscle (upper part of fig. 6), from which it can be stripped as a dissectible layer (lower part of fig. 6); no grossly demonstrable epimysium intervenes between the muscle and the muscle fascia. Laterally the transversalis is perfectly

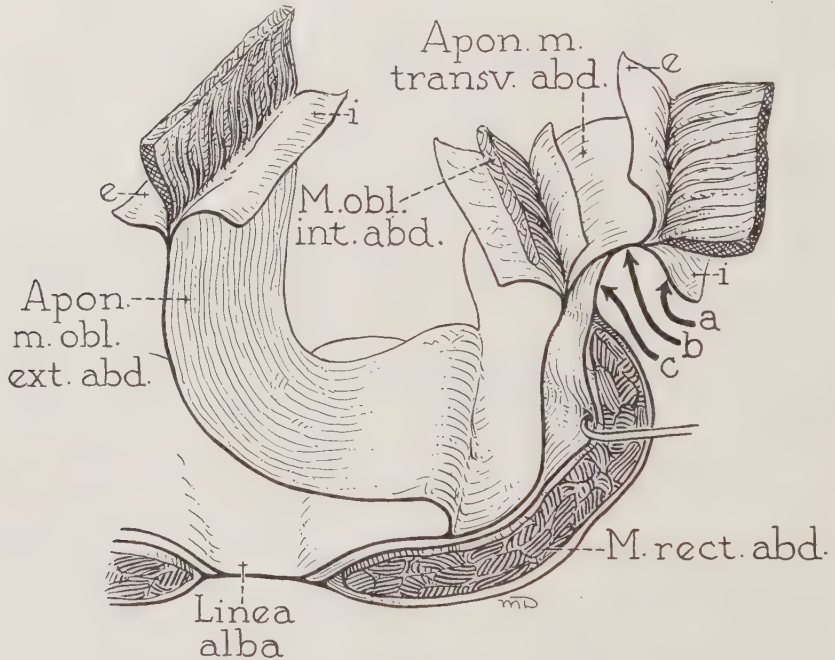


Fig.1 Specimen D. Section demonstrating the constitution of the abdominal wall in the inguino-hypogastric region, at a level inferior to the linea semicircularis. The transversalis fascia (at arrow *a*), joins the aponeurosis (at *b*); from the conjoined layer is derived the fascial investment of the rectus muscle (at *c*). Abbreviations: *a*, external; *i*, internal layer of investing fascia.

continuous with iliac fascia (fig. 6), the latter covering the peritoneal surface of the iliacus muscle in an altogether comparable manner. This continuity establishes the true character of the transversalis fascia, which is that of a simple investing fascia. We find it to be a strong membranous layer, not a fatty one as described by Braus ('21).

Medially, the combined layer (of aponeurosis and investing fasciae) passes anterior to the rectus muscle; as it does so a thin lamina is given off its deep surface (fig. 1, at arrow *c*); passing toward the middle line of the body it splits to form an investment for the rectus muscle. In all but spare and emaciate subjects it is possible to remove this fascia in the form of a definite, unbroken layer. We believe that it should be named for the muscle it covers, and that merely to call it 'transversalis fascia' is as illogical as it would be to apply the latter term to the iliac, the obturator, or the psoas fascia. Its presence may account for the customary statement that, caudal to the semicircular line, the rectus muscle rests upon the transversalis fascia.

Scarcely adequate, then, is the brief statement of Cunningham ('26) and others that the 'transversalis fascia' covers the peritoneal surface of the transversus abdominis muscle and aponeurosis, and that upon it rests the rectus muscle inferior to the level of the semicircular line. It seems essential to distinguish more sharply between fascia and aponeurosis. We would therefore describe these relations as follows: laterally an investing fascia, termed 'transversalis fascia,' clothes the internal aspect of the transversus abdominis muscle (fig. 1, at arrow *a*); near the lateral border of the rectus muscle this fascia (fig. 1, *i*), and the corresponding, though unnamed, external investment (fig. 1, *e*), blends with the aponeurosis of the muscle (fig. 1, at arrow *b*); the aponeurotic plate thus formed by the conjunction of three strata sends off from its deep surface a new, subsidiary leaf (fig. 1, at arrow *c*) which as a 'rectus fascia' divides to send external and internal investing layers about the rectus muscle.

In the inguino-hypogastric area the transversalis fascia and the rectus fascia are clearly distinguishable from the subjacent preperitoneal connective tissue. Although frequently thin on the abdominal parietes the latter is readily traceable into the pelvis as the abundant packing for the viscera and their vessels. In obese subjects the continuity is exceptionally clear, as it also is in those specimens in which a hernia adiposa

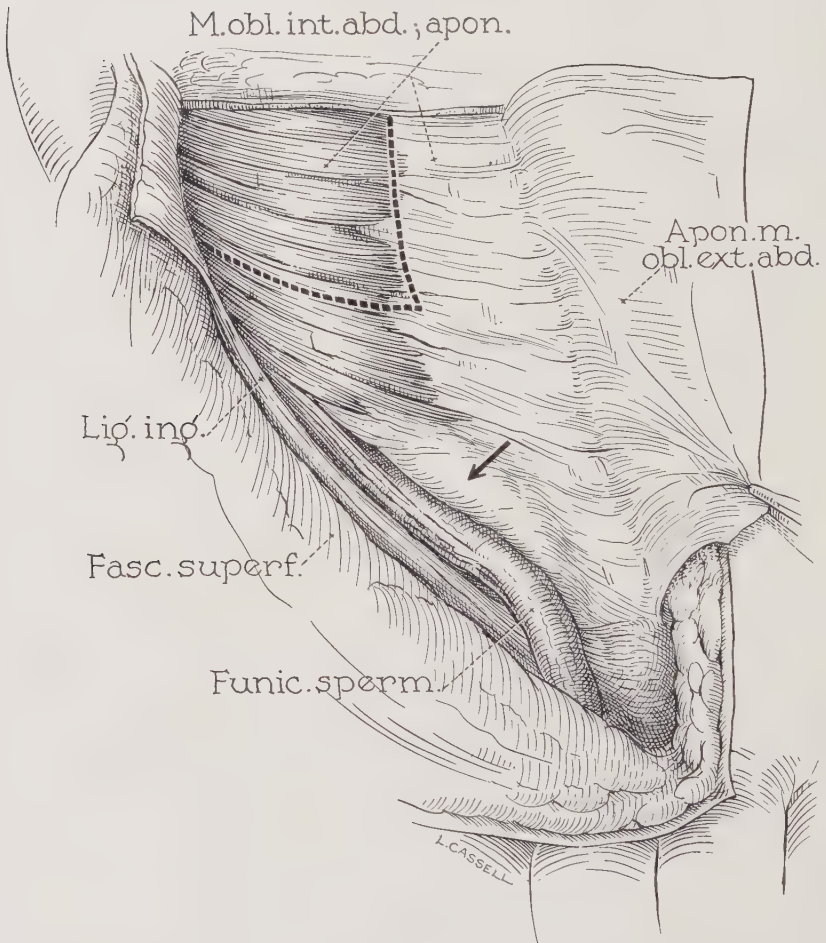


Fig. 2 Specimen E. The aponeurosis of the external oblique muscle turned aside, exposing the internal oblique to the point of union of the aponeuroses of the two muscles. The aponeurosis of the internal oblique in this specimen and in specimen B (fig. 8) is exceptionally broad; in the former the funicular area is aponeurotic, in the latter, muscular. The broken line indicates the junction of the muscular and aponeurotic portions of the subjacent transversus abdominis; the arrow marks the site of a hernia adiposa in the preperitoneal layer of connective tissue. Four-fifths natural size.

occurs (specimen E) to sharpen the contrast between fascia and subserous tissue.

LITERATURE CITED

- BRAUS, H. 1921 *Anatomie des Menschen*. Bd. I. Springer, Berlin.
- CHOUKE, K. S. 1935 Constitution of the sheath of the rectus muscle. *Anat. Rec.*, vol. 61, pp. 341-349.
- CUNNINGHAM, DANIEL 1926 *Textbook of anatomy*, edited by Arthur Robinson, 5th ed., William Wood, New York.
- TESTUT, L. 1921 *Traité D'Anatomie Humaine*, vol. I. Gaston Doin, Paris.

PLATE 1

EXPLANATION OF FIGURES

3 Specimen A. The external oblique aponeurosis has been incised by an oblique cut (to include the superior crus of the ring) and the portions turned aside to expose the internal oblique muscle, its aponeurosis, and the cremasteric fibers; medially, the flap has been reflected to the line of continuity of the two aponeuroses. A small cleft, apparent when the cord is drawn downward, sets off an 'aponeurotic inguinal falx' (above arrow). The line of junction of the aponeuroses of the oblique muscles lies well to the medial side of the lateral border of the rectus muscle (the latter at dotted line), the aponeurosis of the internal oblique thus contributing more extensively to the formation of the inguinal part of the rectus sheath than the external oblique. Three-quarters natural size.

4 Specimen C. A rare condition in which the muscle fibers of the transversus abdominis (ending in an arching border) left exposed the aponeurosis of the transversus abdominis muscle present as a thick aponeurotic layer in the lateral two-fifths of the area, a thin one in the medial three-fifths. Three-quarters natural size.

M. obl. int. abd., apon.

M. rect. abd.

Apon. m. obl.
ext. abd.

3

Fasc. superf.

M.
trans.
abd.

Apon., m.
trans. abd.
(fasc. trans)

Funic. sperm.

M. obl. int. abd.

Apon., m.
trans. abd.
(fasc. trans.)

4

PLATE 2

EXPLANATION OF FIGURES

5 Specimen A (dissection in fig. 3 continued). The internal oblique muscle has now been partially removed to reveal the underlying transversus abdominis muscle. Three-fifths natural size.

6 Specimen A (dissection in fig. 5 continued). The external and internal oblique muscles have been drawn aside and the muscle fibers of the transversus abdominis muscle removed to expose the fascia on the deep aspect of the latter muscle, usually termed the transversalis fascia; it has been freed to the level of the iliac spine; superior to that level it has been left attached to the muscle and turned medially therewith. The layer is continuous with the iliac fascia, the latter exposed by or drawing aside the peritoneal sac. Three-fifths natural size.

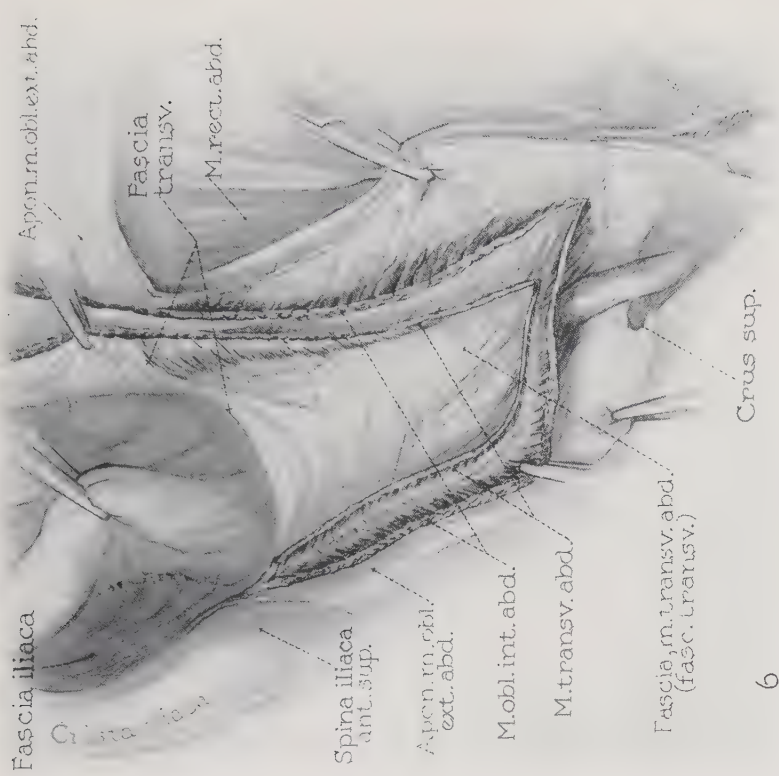
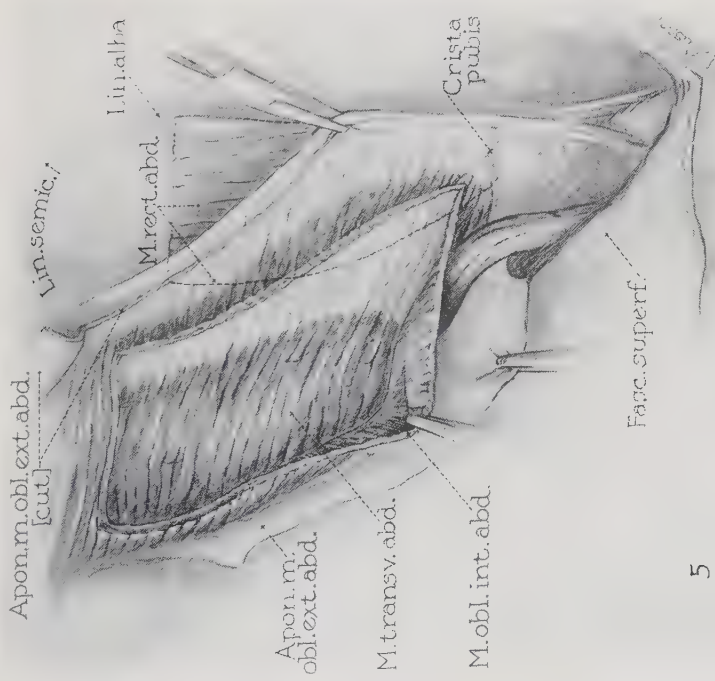
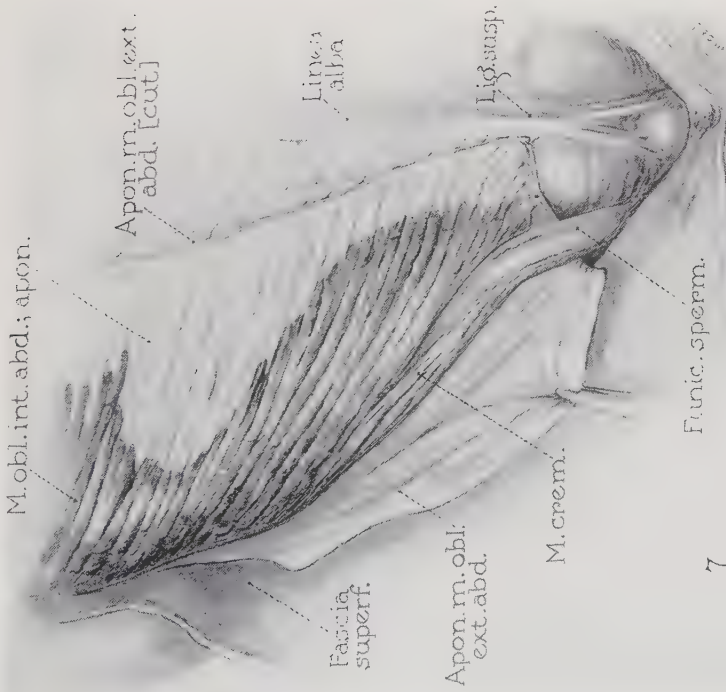


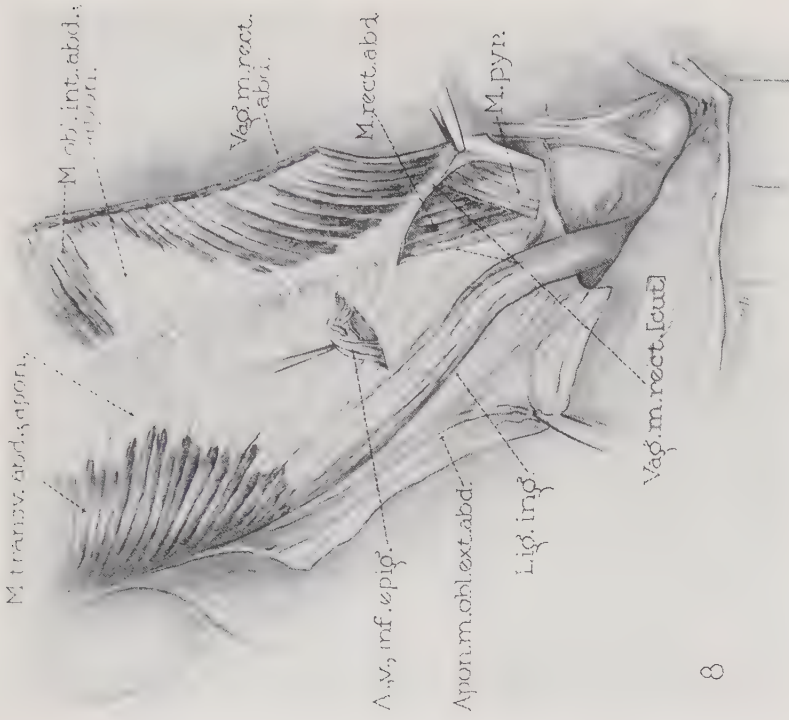
PLATE 3

EXPLANATION OF FIGURES

- 7 Specimen B. Incisions as in figure 3. The aponeurosis of the internal oblique muscle is wider than in specimen A, being more than a broad stripe between muscle fibers and line of continuity with the external oblique. Three-fifths natural size.
- 8 Specimen B (dissection in fig. 7 continued). The aponeurosis of the transversus is unusually broad occupying all of the inguino-hypogastric area except a small portion near the iliac origin. Three-fifths natural size.



7



8

THE VOLUME OF THE PARATHYROID GLANDS OF THE ALBINO RAT

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The volume of the parathyroid glands was studied both to contribute to the knowledge of the anatomy of the rat and to furnish a criterion of size for experimental studies. As far as could be determined from available literature the only similar studies made previously were those by Jackson ('16), Overholser ('29), Jackson and P'an ('32) and Rosof ('34).

The rats used in this work consisted of sixty female and fifty-nine male animals derived from the Wistar strain, grouped as listed in table 2. They were raised in wire bottom cages, fed on McCollum diet I plus 1% of cod liver oil, and furnished fresh water daily. The rats were killed with chloroform at 28 days, 3 months, or 6 months of age. From some the thyroid and parathyroids were removed together with adjacent portions of the trachea and larynx; from others the thyroid, with the parathyroids, was carefully dissected free from surrounding structures. The glands were fixed immediately in Bouin's fluid, Bensley's modification of Helly's fluid, or Regaud's fluid, care being taken to assure comparable fixation in each group of rats. Washing, dehydration, clearing, and embedding were done in the usual manner. The glands were sectioned serially at a thickness of either 8 or 10 μ , and stained with Harris' hematoxylin and eosin Y. The volume of the parathyroids was determined by the paper outline method, employing a Leitz-Edinger projection apparatus and tracing the sections at a magnification of 97 diameters on 'Artesian Linen Record' paper. The drawings were cut out and weighed, and having previously determined the weight of a known area of paper, the volume was calculated. This

method is similar to the one employed by Overholser ('29) except that he determined surface area of the outline drawings by means of a planimeter.

The task of estimating volume by this method is a considerable one if every section is drawn. It was decided, therefore, to determine how few sections could be drawn and still obtain results similar to those derived from the use of every section. For this purpose thirty parathyroid glands were employed. Every section was outlined and the drawing numbered. The volume of each parathyroid was then determined making use of drawings of every section and every second, fourth, sixth, eighth and tenth sections. The results were

TABLE 1

Comparison of mean weights of paper drawings, made at various intervals, of the same series of thirty parathyroid glands

SECTION INTERVAL	MEAN AND S.D.	DIFFERENCE ¹ AND P.E.	S.R.
μ	gm.		
10	8.3841 $\pm$ 1.6971		
20	8.3990 $\pm$ 1.6939	0.0149 $\pm$ 0.3003	0.05
40	8.3827 $\pm$ 1.7100	0.0014 $\pm$ 0.3017	0.005
60	8.4254 $\pm$ 1.7066	0.0413 $\pm$ 0.3015	0.14
80	8.3376 $\pm$ 1.7150	0.0465 $\pm$ 0.3022	0.15
100	8.3047 $\pm$ 1.7467	0.0794 $\pm$ 0.3050	0.26

¹ Difference between mean value of paper weight of drawings made at 10 μ intervals and that of drawings made at successively greater intervals.

compared statistically. In determining the significance of differences, the correlation between results from the same series of glands was a factor that had to be considered. For this reason the following formula was used in obtaining the probable error of the difference between the means:

$$P.E.(\bar{x} - \bar{y}) = \frac{0.6745}{\sqrt{N}} \sqrt{\sigma_x^2 + \sigma_y^2 - 2 r_{xy} \sigma_x \sigma_y}$$

Table 1 contains the results of this study.

The mean value obtained by outlining sections at 100 μ intervals showed the greatest deviation, 1.0%, from the value obtained by drawing every section. The difference was not

statistically significant. Drawing sections at 100 μ intervals should suffice, which means that the volume of a parathyroid gland may be determined by making from six to ten outline drawings. Since the glands used for this study were from 6-month male rats, the possibility that the above conclusion was not true for smaller glands from younger rats could not be overlooked. It was decided, somewhat arbitrarily, to estimate the volume by outlining sections at intervals of 40 μ , thus creating a margin of safety.

RESULTS

a. Volume

In table 2 are listed the mean values of the estimated volume of the parathyroid glands at various ages for both sexes.

TABLE 2
Body weight, volume and relative volume

SEX	AGE	NUMBER	BODY WEIGHT	MEAN VOLUME, S.D.	DIFFERENCE, P.E.	S.R.	PER CENT DIFFERENCE	RELATIVE VOLUME	PER CENT DIFFERENCE
F	28 days	21	gm. 47.9	cu.mm. 0.0653 ± 0.0141				0.1364	
M	28 days	20	53.5	0.0506 ± 0.0100	0.0147 ± 0.0025	5.8	29.1	0.0945	44.3
F	3 months	20	164.5	0.1635 ± 0.0316				0.0994	
M	3 months	18	240.8	0.1437 ± 0.0316	0.0198 ± 0.0068	2.9	13.8	0.0596	66.8
F	6 months	19	213.1	0.2450 ± 0.0489				0.1149	
M	6 months	21	299.0	0.2217 ± 0.0529	0.0233 ± 0.0107	2.1	10.5	0.0741	55.1

At 28 days of age female rats had parathyroids 29.1% larger than the male glands; at 3 months, 13.8% larger; and at 6 months, 10.5% larger. The difference between means was statistically significant in the 28-day and 3-month groups, and probably so in the 6-month group.

b. Relative volume

A ratio between body weight in grams and parathyroid volume in cubic millimeters was made by dividing volume by weight and multiplying by 100. Data are listed in table 2. The female rats were found to have relatively more parathyroid gland than the males in every age group, the per cent excess being: 28-day, 44.3% ; 3-month, 66.8% ; 6-month, 55.1%.

c. Growth changes in volume

In female rats the volume of the parathyroid glands increased 150.4% from 28 days to 3 months of age, 49.8% from 3 to 6 months, and 275.2% from 28 days to 6 months. Increases of the volume of the parathyroid glands of male rats were 184.0% from 28 days to 3 months, 54.3% from 3 to 6 months, and 338.1% from 28 days to 6 months. Either growth of volume was more rapid in the male rats or more precocious in the females. However, the relative volume of the parathyroids increased less in the male rats during this age period. In the female rats there was a decrease of 15.8% in relative volume from 28 days to 6 months; in males the decrease was 21.6%. It is of interest to note that in both female and male rats the decrease of relative volume took place between 28 days and 3 months of age, while between 3 and 6 months the relative volume increased. From these data it is apparent that over the period studied the growth curve of the parathyroids did not parallel that of body weight.

d. Comparison of right and left glands

Only in those cases in which the thyroid and parathyroid glands were removed together with adjacent portions of trachea and larynx was it possible to determine with certainty which was right and which left of the parathyroids. Since this manner of removal was not used with 3- and 6-month male rats, these groups were omitted from consideration.

The data in table 3 show that in all the groups studied except the 28-day female the left parathyroid gland was larger than the right, exceeding it in volume by 5.7% in the 3-month females, by 8.9% in the 6-month females, and by 2.4% in the 28-day males. In the 28-day females the right gland was 0.3% larger than the left. Although none of these differences were statistically significant, the greater frequency of larger left glands cannot be overlooked. In each group the varia-

TABLE 3
Comparison of right and left glands

GROUP		NUM- BER	MEAN VOLUME, S.D.	DIFFER- ENCE, P.E.	S.R.	PER CENT DIFFER- ENCE	C.V.	DIFFER- ENCE, P.E.	S.R.
			<i>cu.mm.</i>						
Female, 28-day	Right	21	0.0327 ± 0.0100				30.58		
	Left	21	0.0326 ± 0.0051	0.0001 ± 0.0016	0.1	0.3	15.64	14.94 ± 3.94	3.8
Female, 3-month	Right	20	0.0795 ± 0.0244				30.69		
	Left	20	0.0840 ± 0.0223	0.0045 ± 0.0049	0.9	5.7	26.54	4.15 ± 4.80	0.9
Female, 6-month	Right	19	0.1173 ± 0.0360				30.69		
	Left	19	0.1277 ± 0.0223	0.0104 ± 0.0065	1.6	8.9	17.46	13.23 ± 4.27	3.1
Male, 28-day	Right	18	0.0249 ± 0.0079				31.60		
	Left	18	0.0255 ± 0.0062	0.0006 ± 0.0015	0.4	2.4	24.47	7.13 ± 5.00	1.4

bility of volume of the right gland was greater than that of the left, the difference between coefficients of variation being significant for the 28-day and 6-month female groups. The left gland, it may be concluded, exhibited a tendency to be larger and less variable in volume than the right.

DISCUSSION AND SUMMARY

By trial it was found that, using the paper outline method to determine volume, drawing as few as every tenth of 10μ

sections gave results not significantly different from those obtained by drawing every section.

At 28 days, 3 months and 6 months of age female albino rats of the Wistar strain were found to have larger parathyroid glands than male rats. The differences were significant in the first two groups and probably so in the third. The relative volume of the parathyroid glands of the female was approximately twice that of the male. Jackson ('16) measured the length, and greatest and least cross section diameters of the parathyroid glands of albino rats and concluded that they appear to be relatively larger in the female. Overholser ('29) presented data on the size of the parathyroid glands of male albino rats 60, 75 and 90 days old, in terms of the surface area of the sections outlined. Calculation of volume in cubic millimeters from his data gave the following values for controls: 60-day males, 0.1653; 75-day males, 0.2384; 90-day males, 0.2331. The value obtained from his data for 90-day males, 0.2331 cu.mm., is 62% greater than the volume of 0.1437 cu.mm. found for 3-month males of the present study. This difference becomes more striking on consideration of the fact that his 90-day males were 41% lighter in average body weight than the 3-month males of the present series. Jackson and P'an ('32) determined the volume of the parathyroids of five female and six male albino rats 8 months old and found the female glands absolutely as well as relatively larger. Rosof ('34), using the method of Overholser, determined but did not publish data concerning the size of the parathyroid glands of male and female albino rats. Our observations confirm and extend these earlier reports. It is interesting to note that Pappenheimer and Wilens ('35) found the parathyroid glands of female human beings during the age period of sexual activity weighed more than those of adult males. Morgan ('36) believed that his data, measurements of human parathyroid glands, indicated a weight variation similar to that recorded by Pappenheimer and Wilens. It seems reasonable to assume that the larger parathyroid glands of the female albino rat are associated with the special functions of gestation and lactation.

There was an indication, though the differences were not all significant, that the left parathyroid was usually the larger and less variable in volume of the pair. It may be a coincidence or it may be significant that the left adrenal gland is usually larger than the right.

During the period from 28 days to 6 months of age the parathyroids of male rats increased more in absolute but less in relative volume than those of the females, which indicated a sex difference in growth. In neither group did parathyroid volume growth parallel body weight growth.

LITERATURE CITED

- JACKSON, C. M. 1916 Effects of inanition upon the structure of the thyroid and parathyroid glands of the albino rat. *Am. J. Anat.*, vol. 19, pp. 305-352.
- JACKSON, C. M., AND M. T. P'AN 1932 The effects of dietary deficiency of iodine upon the thyroid and parathyroid glands in the rat. *Endocrinology*, vol. 16, pp. 146-152.
- MORGAN, J. R. E. 1936 The parathyroid glands. I. A study of the normal gland. *Arch. Path.*, vol. 21, pp. 10-26.
- OVERHOLSER, M. D. 1929 The effect of castration upon the size of the parathyroid glands and upon the susceptibility to tetania parathyropriva in the albino rat. *Anat. Rec.*, vol. 41, pp. 303-321.
- PAPPENHEIMER, A. M., AND S. L. WILENS 1935 Enlargement of the parathyroid glands in renal disease. *Am. J. Path.*, vol. 11, pp. 73-91.
- ROSOF, J. A. 1934 An experimental study of the histology and cytology of the parathyroid glands in the albino rat. *J. Exp. Zool.*, vol. 68, pp. 121-165.

BOOK REVIEW

A STEREOSCOPIC ATLAS OF THE CHICK. By J. A. Long, Associate Professor of Embryology, and Paul L. Burlingame, Research Associate in Zoology, University of California, Berkeley. California Laboratory Supply Co., 928 Central Bldg., Los Angeles, Calif., 1937. 113 stereoscopic plates (3.5×5.5 in.) with folding stereoscope. Price \$7.35.

Since the fourth century, B.C., when a pioneer embryologist of the Hippocratic School advised those who would know something about the human fetus to examine the eggs of a brooding hen—there to “find everything as I say, in so far as a bird can resemble a man”—the chick embryo has been of great interest to students of medicine: witness the significant studies of such physicians (or anatomists) as Aldrovandi, Coiter, Fabricius, Harvey, Malpighi, John Hunter, Wolff, v. Baer, Pander, His and others too numerous to mention. Although, more recently, anatomists have tended to emphasize the differences between the chick and the human embryo, the fact remains that the chick still provides the most available and perhaps the simplest laboratory approach to an understanding of the differentiation of germ layers in warm-blooded vertebrates. Thus any new method of displaying these relations should be welcomed by teachers of embryology in premedical and medical courses.

Such a new contribution is now available in the form of a stereoscopic atlas based largely upon gross dissections carried out under the binocular microscope—a technique familiar to students of embryology but not hitherto used on a large scale to demonstrate the development of the chick. In a personal communication Professor Long writes that the use of this atlas, in his class of some 200 students, has greatly simplified the study of serial sections and thereby shortened the period required for laboratory work.

The first twenty-one plates—covering a graded series of embryos ranging from primitive streak to 4- to 5-somite stages (16 to 24 hours of incubation) consist of dorsal and ventral surface views of unstained opaque embryos together with views of stained and cleared specimens of corresponding age.

In the next forty-three plates—covering the 5, 7, 10, 13, 18, 20, 21 and 24-somite stages—views of the surface are supplemented with views of dissections of unstained embryos.

Beginning with Plate 65 the dissections become more numerous and elaborate. Thus sixteen plates are devoted to the 30-somite stage (57 hours), ten to the 37- to 39-somite embryo (84 hours) and eight to the 'Fourth day.'

The fourth set of plates (99 to 113) deal with the circulatory system in a graded series of cleared embryos injected with India ink.

The last plate, displaying a very elaborate and detailed dissection of a 15-day rat embryo, is introduced as a sample of what might be expected should there be a demand for similar stereoscopic atlases of mammalian and urodele embryos. Because of the excellent quality of this work, the writer of the review hopes that such deserved encouragement will be forthcoming.

Returning to the atlas of the chick, one is impressed not only with the number of special dissections but also with the superb technique, both manual and photographic, that we have learned to expect from the skilled hands of Professor Long and his associates. Especially well displayed are such structures as Rathke's pocket, the heart and pericardium, the subcephalic pocket, the optic vesicle and chorioid fissure, the pharynx (both its internal and external topography) and the cavities of the brain—all of them structures that students find difficult to interpret in sections. Only in one region do the authors seem not to have fully realized the possibilities, namely in the details of origin of the hind-gut and the isolation of the tail-bud mass through differentiation of the primitive streak at levels both caudad and cephalad to Hensen's node. In this caudal region one should distinguish between the anal diverticulum—a temporary crescent-shaped invagination of the entoderm, which grows up into the post-caudal portion of the primitive streak to form the anal plate—and that elevation of the entoderm at the site of the neurenteric canal which first creates the hind-gut. Continued over-growth of the tail-bud mass, with the coincident moving forward of the ventral lip of the hind-gut, then completes the flattening out of the anal diverticulum and, in so doing, creates an elongated ventral floor from which the allantois will soon invaginate.

Also the writer would like to have seen a series of photographs displaying the vestigial gill filaments of the third and fourth pharyngeal arches, the first appearance of which—in the form of an external hillock—is shown in several plates, particularly in no. 94, just above the end of the guide line labelled, '3rd visceral groove.' But these comments are to be considered as suggesting future additions to the series rather than as subtracting from the merits of this excellent 'guide to the perplexed.'

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NEW BOOKS AND PUBLICATIONS

MEDICO-LEGAL ASPECTS OF THE RUXTON CASE, by John Glaister and James Couper Brash. 1937. Size, $7\frac{1}{4} \times 10$ inches. Pages xvi + 284. 172 illustrations. Index. Buckram. Price, \$6.00. William Wood and Company, Baltimore.

Readers will doubtless recall the Ruxton Case which created such a sensation in the newspapers, when on the afternoon of September 29, 1935, mutilated human remains were discovered in the bed of a stream near Moffatt, a small town in Dumfriesshire, Scotland. This volume deals with the medico-legal aspects of the case, which is without parallel in criminal records. Scientists who are professionally interested in methods employed in preparation of medical and technical evidence will follow with interest the detailed accounts of each step of the process whereby scattered fragments of flesh and bone were assembled, preserved, arranged and definitely identified as the bodies of Mrs. Ruxton and her maid, Mary Rogerson. The nature and extent of the mutilation of the two victims provided a problem of reconstruction which demanded, for its solution, anatomical work in detail not hitherto required in such cases. The numerous photographic illustrations aid materially in making the book a complete record of the scientific aspects of the Ruxton Case.



A CASE OF THORACOPAGUS MONOSYMMETROS

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NINE FIGURES

In view of the modern conception that the typical thoracopagus represents partial fusion of two inner cell masses within a single blastocyst, and that the twins so formed have therefore an identical genetic constitution, the present specimen offers a unique opportunity to analyze such variations in structure as are of purely environmental origin.

This specimen was delivered to the anatomy department of Aberdeen University by Dr. James S. Stewart. The mother, a healthy multipara of 35 years of age, had had two previous pregnancies. The first pregnancy terminated 10 years ago with the birth of a normal child but in the course of the pregnancy the patient suffered from severe eclampsia. The second pregnancy was normal in all respects and a full time healthy child was born. The third pregnancy was also normal but the monster about to be described was born without much difficulty. The monster was delivered at full time but was stillborn. The patient had no previous malformed children and there was no history of twins or of congenital abnormalities on either the father's or mother's side of the family.

EXTERNAL APPEARANCES

The monster (fig. 1) consists of two male infants lying with the ventrolateral surfaces opposed and joined together by thorax and abdomen. There are separate heads, upper and lower limbs and genitalia for each half of the monster but the two halves seem on superficial inspection to have a common thorax and abdomen. They do not face one another exactly as

in thoracopagus disymmetros so that it is possible to distinguish a right and left twin. There is a single placenta of normal size and appearance and one umbilical cord running toward the right side of the monster. Each foetus is 35 cm. from vertex to sole but the head of the foetus on the left side is slightly smaller than that on the right.

Umbilical cord

The cord is 58 cm. long from the placenta to the point where it is attached to the foetus on the right side. There are two umbilical arteries and one umbilical vein in the cord. The cord is common to both halves of the monster. It passes first to the one on the right side and then passes round between the right infant and the viscera protruding from the common umbilical opening and following the edge of that opening enters the foetus on the left toward the posterior aspect of the umbilicus. There are two arteries and one vein in the umbilical cord but the distribution of these is curious. Two umbilical arteries arising from the internal iliac arteries emerge from each foetus. Before they reach the umbilicus each pair of arteries unites to form a single trunk producing the two umbilical arteries present in the cord. The umbilical vein enters the foetus on the left side.

Umbilical opening

Projecting from the large common umbilical opening, and when presented here partially covered by amnion, there are several loops of bowel and the greater part of the liver. The liver is in two parts a large lobe behind and a smaller one in front completely filling the umbilical orifice. Between these two lobes a mesentery descends supporting loops of bowel. In the center there is a portion of bowel descending ventrically into a large dilated saccular portion; which was ruptured during parturition, from which two other loops extend laterally and join the caecum on each side, and the ascending colon passes upward into the abdominal cavity. On either side of the umbilical opening between the liver and the abdominal wall

there is a short dependent loop of bowel. These loops, especially that on the right, are distended with meconium, are much wider than the remainder of the large bowel, and present the appearance of being partially obstructed.

External genitalia

The external genitalia are normal in appearance but both infants have bilateral undescended testes.

Anus

Normal perforate anus present on both sides.

Limbs

The limbs of the infant on the right side are normal. The right arm and leg of the left infant are normal but the left foot has only the great and second toes present with a cutaneous appendage representing a third toe halfway along the lateral side of the foot. The left upper extremity presents a congenital club hand deformity with absence of the radius and thumb.

X-RAY EXAMINATION

This shows absence of the sternum. The left upper limb of the foetus on the left side exhibits absence of the radius and a short thickened ulna which is bowed posteriorly. Also in the hand only four metacarpals are present. In the left foot of the same foetus one finds only two metacarpals and two sets of phalanges present. After injection of barium cream through the umbilical vein the fact that there is one heart common to both infants is demonstrated.

ABDOMINAL VISCERA

Alimentary canal. The pharynx and oesophagus are normal on both sides and the oesophagus pierces the diaphragm and enters the stomach. The two stomachs lie in grooves one on either side of the liver (fig. 3), where the narrow bridge

unites the two halves of that organ. The stomach on each side passes into the duodenum which is of almost the same caliber as the stomach. The upper parts of the duodena are separate and then unite, and at right angles to the point of union a vertical loop of bowel descends between the two lobes of the liver (fig. 4), and becoming dilated terminates below in a large dilated saccular portion of gut (fig. 2). This portion presents ridges and depressions on its surface and looks as if several coils of bowel had become adherent but the interior consists of a single chamber. From this sac on either side the terminal ileum extends laterally and enters the caecum. An appendix is present on either side. The large bowel ascends into the abdomen and then sends down the loop on either side which has already been described as showing partial strangulation (figs. 1, 2). The bowel then returns to the abdomen and continues to the rectum.

Liver. The liver which protrudes from the umbilical opening (figs. 3, 4), is formed by the union of two main lobes. The smaller portion is anterior and the larger posterior, the two being united by a bridge of liver tissue. The anterior lobe is about half the size of the posterior lobe and between the two there is a deep cleft. On the two flat opposed visceral surfaces there are two small collapsed gall bladders (fig. 3). The

Fig. 1 Photograph of the undissected monster. A, amnion covering anterior lobe of liver; B, left upper limb showing congenital club hand deformity; C, posterior lobe of liver; D, saccular portion of small intestine; E¹ and E², obstructed loops of large intestine.

Fig. 2 Photograph of alimentary canal. A, oesophagus; B, stomach; C, duodenum; D, saccular portion of small intestine; E¹ and E², obstructed loops of large intestines; F, rectum; G, appendix.

Fig. 3 Photograph of alimentary canal placed in relation to the posterior lobe of the liver. A, posterior lobe of liver; B, cut surface of isthmus connecting anterior and posterior lobes of liver; C, gall bladder.

Fig. 4 Photograph of alimentary canal placed in relation to both lobes of the liver. A, anterior lobe of liver; B, posterior lobe of liver; C, common portion of small intestine descending between the lobes of the liver.

Fig. 5 Photograph of thoracic viscera (anterior aspect). A, thyroid gland; B, thymus gland; C, left lung of foetus on right side; D, right lung of foetus on right side; E, left lung of foetus on left side; F, left auricle of the right half of the heart; G, ventricular portion of right half of the heart; H, ventricular portion of left half of the heart.

FIG 2.

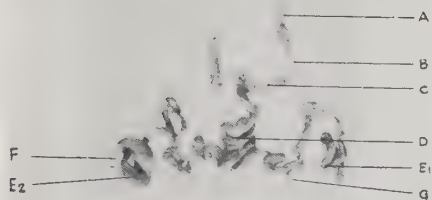


FIG 3.

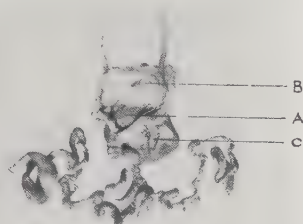


FIG 1.



FIG 4.

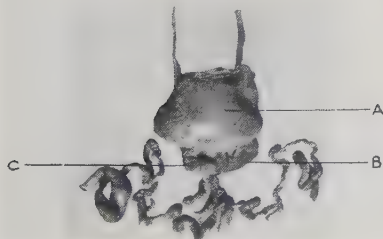
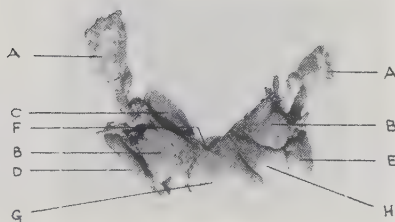


FIG 5.



abdominal part of the oesophagus, the cardia and the upper part of the body of the stomach lie in grooves one on either side of the isthmus of liver tissue (fig. 3). The pyloric portion of the stomach and the duodenum as far as the ampulla of Vater lie in the transverse fissure between the two lobes. The stomachs and duodena are suspended by a mesentery from the transverse fissure. Both lobes of the liver are grossly deformed and none of the normal subdivisions of the liver can be identified with certainty. There are three hepatic veins joining right inferior vena cava. The left inferior vena cava does not traverse liver substance and receives no hepatic veins.

Spleen. Right spleen is normal. The spleen in the left infant is lobulated consisting of seven lobules of varying size united together only by branches of the splenic artery and vein.

Kidneys, suprarenals, ureters and bladders. In the right foetus the kidneys, suprarenals, ureters and bladder are all normal. In the left foetus the right kidney, suprarenal and ureter are normal. The left suprarenal is normal but the left kidney is hydronephrotic and there is a hydroureter, the dilation extending down almost to the ureteral opening of the bladder where, however, the ureter suddenly narrows to normal dimensions. There is one solitary cyst on the upper pole of this kidney containing a quantity of blood clot and some watery fluid similar to that present in the hydronephrotic sac. There is a definite constriction in the ureter at the pelvi-ureteric junction. The left inferior vena cava crosses the pelvis of the left kidney of the foetus on the left side and there is a definite impression on the pelvis due to the pressure of this vessel but in view of the dilated conditions of the ureter as well as the imperfect degree of constriction of the pelvis, it almost certainly has nothing to do with the hydronephrosis and hydroureter. The bladder on this side presents no abnormality.

Testes. There are abdominal organs in both infants lying at the lower pole of the kidneys suspended by a mesorchium.

Diaphragm. This structure is common to the two halves of the monster. Just to the left of the midline and in the anterior portion of the diaphragm there is a round aperture about 1 cm. in diameter. This is probably an unclosed pleuro peritoneal canal. There is no herniation of viscera.

THORACIC VISCERA

On both sides there is a well-developed thyroid and thymus gland and both sets of lungs are normal in appearance exhibiting the standardized lobulation. The lungs are collapsed and have never functioned. All these viscera are, however, smaller on the left side than the corresponding ones on the right (figs. 5, 6).

The heart

The heart is common to both halves of the monster. On the anterior aspect of the undissected heart there is a fissure dividing the ventricular portion into two halves (fig. 5). From this portion of the heart the aorta and pulmonary arteries arise. On the postero superior aspect of the heart there is a large thin walled chamber on the middle of the upper surface of which is perched what appears, on external examination, to be another chamber of the same thin walled auricular type (fig. 6). A superior and inferior vena cava opens into the large posterior chamber of the heart. The pulmonary veins open into the superior vena cava on either side. The thin walled auricular portion of the heart has a very smooth wall apart from two small auricular appendages on either side (fig. 7). It is constricted in the center giving the whole cavity a dumb-bell shape and in the floor there is a small flap of endocardium as if some attempt at partition formation had been made.

There are three openings leading from the chamber into other portions of the heart (fig. 7). In the roof of the right half of the chamber there is a large oval aperture unguarded by valves. This aperture leads into the small thin walled cavity situated on the upper part of the heart. In the floor

of each half of the large auricular chamber there is a tricuspid opening leading into the right ventricle of each side of the heart. The small upper chamber of the heart is very thin walled and communicates with the right half of the postero superior auricular chamber through the large oval opening described above. It opens also into the left ventricle of the right side of the heart through a bicuspid opening. It has no connection with the left half of the heart at all (fig. 8). The ventricular portion of the heart consists of four chambers. There is a right and a left ventricle on either side which are incompletely separated from one another while the two pairs of ventricles are quite distinct. The walls of the ventricles exhibit nothing unusual and the mitral and bicuspid valves have cusps conforming to type. On either side the left ventricle gives rise to the aorta and the right ventricle to the pulmonary artery, but owing to the failure of complete development of the interventricular septum these arterial trunks are not completely separated at their origin and blood has therefore access to the aorta from both ventricles (fig. 9).

Fig. 6 Photograph of thoracic viscera (posterior aspect). A, left superior vena cava; B, right descending thoracic aorta; C, common sinus venosus; D, left auricle of right half of the heart; E, right lung of left foetus; F, left lung of right foetus; G, right lung of right foetus.

Fig. 7 Photograph of the interior of the common sinus venosus. A, rod passing through foramen ovale into the left auricle of the right half of the heart; B, rod passing from sinus venosus through the tricuspid opening into the right ventricle of the right half of the heart; C, rod passing from sinus venosus through tricuspid opening of the left half of the heart; D, superior vena cava; E, right auricular appendage of left half of heart; F, right auricular appendage of right half of heart.

Fig. 8 Photograph of anterior aspect of heart showing the interior of the ventricles on the left side. A, rod behind interventricular septum of left side and passing into aorta above; B, pulmonary artery; C, interventricular septum; D, rod from left auricle to left ventricle of right side; E, right pulmonary artery; G, rod from right ventricle of right side to pulmonary artery.

Fig. 9 Photograph of anterior view of heart showing the interior of the right ventricle of the right half of the heart. A, rod from right ventricle to pulmonary artery; B, rod from left ventricle to aorta; C, bulbar ridge meeting upper edge of interventricular septum D.

FIG 6.

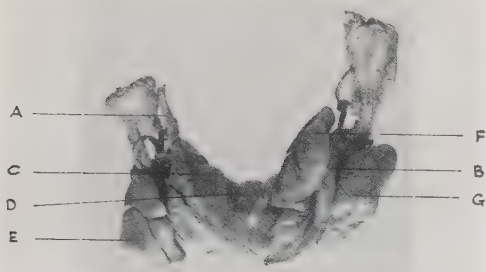


FIG 7.

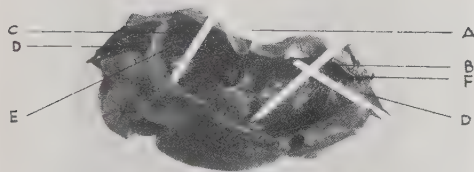


FIG 8.

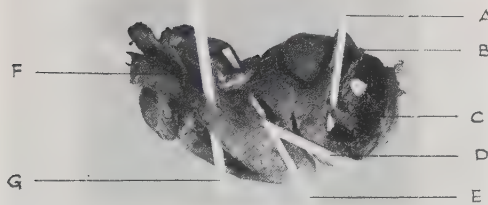
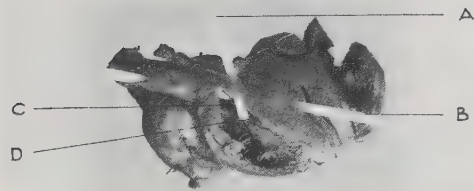


FIG 9.



Explanation of findings in the heart

This monster consists of a foetus in which at an early stage certain structures have been duplicated while others have remained single. The sinus venosus has remained a single structure because of the presence of the uninterrupted flat smooth walled area on the back of the right auricular chamber. Thus we have duplicated venae cavae opening into a common sinus venosus and the two right auricles are represented by the two auricular appendages. The foramen in the interauricular septum on the right side (fig. 7), is the foramen secundum or foramen ovale of that side. The right half of the heart has four chambers. It shares the common sinus venosus and has a small right auricular appendage of its own. The small chamber on the upper surface of the heart is the left auricle of the right half of the heart. There are also a right and a left ventricle, the left ventricle communicating with the left auricle through the mitral valve and the aorta arising from the left ventricle (fig. 8). The right auricle communicates with the right ventricle through the tricuspid valve and the pulmonary artery arises from this chamber. The left half of the heart has three chambers. It shares the sinus venosus and there is a small auricular appendage. There is no left auricle. The right ventricle communicates with the sinus venosus through the tricuspid opening and the aorta arises from the left ventricle while the pulmonary artery which on this side is very small comes from the right ventricle. The two ventricles in the left half of the heart are separated below by an interventricular septum (fig. 8). The bulbar ridges on this side separate the aortic from the pulmonary part of the bulbus cordis. The reason for this conclusion is that below the aortic and pulmonary valves there is complete separation of the two vessels. The bulbar ridges though fused with one another have not united with the upper border of the interventricular septum so that there is free communication between the right and left ventricles over the upper part of the septum.

On the right side the interventricular septum is again present and the bulbar ridges also (fig. 9). These ridges pass down and coalesce with the upper border of the interventricular septum but they have remained unfused. On this side the aortic and pulmonary valves are at the lower end of the separated aorta and pulmonary artery showing that there has been a complete failure of fusion of the bulbar ridges and there is a bulbus cordis common to both aorta and pulmonary artery. This state of affairs differs from that found on the left side where partial fusion of the bulbar ridges above has taken place. On the right side therefore, we again have a free communication over the upper edge of the interventricular septum between the right and left ventricles. The interventricular septum on the right side is very much higher than that on the left and in view also of the fact that the bulbar ridges pass down and coalesce with the upper part of the septum it is possible that the septum as seen in the specimen may have been contributed to in its upper part by fusion of the lower parts of the bulbar ridges. We have, therefore, a partial fusion of the bulbar ridges on both sides of the heart, but on the left side the fusion has occurred above, while on the right side—at least judging from the height of the interventricular septum—it has occurred below.

Course of the blood through the heart

The blood enters the right auricular chamber from the venae cavae. A proportion of this passes through the foramen ovale to the left auricle and thence to the left ventricle of the right half of the heart via the bicuspid opening. The blood in the left ventricle passes via the aorta to the general circulation. The remainder of the blood in the right auricle passes through one or other of the tricuspid openings to the right ventricle of both sides. From the right ventricle it passes through the pulmonary artery and some may escape to the aorta on account of the deficiency of the interventricular septum. On the left side of the heart because of the absence of a left auricle all the blood passing to the ventricles has to

pass via the tricuspid opening so that the defect in the inter-ventricular septum insures that blood will reach the aorta on this side.

BRAIN

The lateral ventricles of both brains are full of blood clot but otherwise there is no abnormality discernible by naked eye.

DISCUSSION OF FINDINGS

Because of the presence of the single umbilical cord and placenta it can be inferred that the monster is the result of the splitting of a single fertilized ovum rather than the partial fusion of two fertilized ova.

Duplication of structures has occurred at the head and tail ends and on the dorsal aspect, while certain ventral structures have remained single and common to both twins.

The limbs, head, central nervous system, respiratory organs, and, to a certain extent, the cardiovascular organs and alimentary canal and its derivatives, have been duplicated. The duplication of the heart is incomplete, while a portion of the alimentary canal, and the diaphragm, have remained single. From the presence of a complete nervous system in each twin it appears that duplication must have commenced in the embryonic plate before the appearance of the primitive streak, and consequently before the development of any vascular formation in the embryo proper. The yolk sac being single has lead to the formation of only one set of vitelline veins and consequently only one set of hepatic veins which joins the inferior vena cava of the right twin.

The involvement of the various components of the cardiovascular system in this process of duplication varies according to whether these parts are ventrally or dorsally placed. These parts developing toward the dorsal aspect are duplicated while those on the ventral surface have remained single. Thus, as in a normal embryo, there would be a single vitelline system and two umbilical veins running from the body stalk forward to the anterior end of the embryonic plate and lying near the edge of the amnion. These umbilical veins normally

turn dorsally each becoming continuous with a dorsal aorta. However, in this case we have duplication of the structures in the dorsal region of the embryo so that each umbilical vein at this stage bifurcates to form two aortae which subsequently join to form a single vessel running into the body stalk. The normal number of vessels in the umbilical cord is thus maintained. That this has been the state of affairs is supported by the fact that the two umbilical arteries found in the pelvis of each twin join to form a single vessel before reaching the umbilicus.

The heart tube is normally formed by the fusion of the umbilical veins as they bend dorsally to be continuous with the aortae. In this case fusion of the ventral ends of the loop has occurred to form the common sinus venosus while the dorsal ends of the loops have remained unfused to produce the auricles and ventricles of the duplicated portions of the heart.

The portion of the embryo which has remained unduplicated is the yolk sac, a part of the mid-gut, and the septum transversum. The fore-gut is double and consequently there are two biliary systems. The presence of a single liver is misleading as the liver found is the result of the fusion of two liver buds due to proximity and having grown into a single septum transversum. The presence of the two biliary systems indicates that the composite liver has arisen as two liver buds from the duplicated fore-gut. The mid-gut which commences just below the duodenal ampulla and continues to the splenic flexure is single in its upper part and duplicated below. The single portion extends down to a point in the terminal ileum where the dilated sac, presenting the appearance of being formed of fused loops, is to be found. This point below which the mid-gut is duplicated is probably that point at which the single vitelline duct joined the bowel. The saccular portion of the bowel suggests fusion of the loops of the cranial half of the umbilical loop of bowel and this further supports the contention that the mid-gut remained unduplicated down to the vitello-intestinal duct.

This monster represents an imperfect attempt at twin formation at or before the formation of the primitive streak and before development of the neural groove. Duplication has occurred in all regions except at the anterior end of the mid-gut and in the septum transversum the duplication of certain portions of the heart being produced by a lack of fusion of dorsal ends of the primitive cardiac loops.

SUMMARY

A case of *thoracopagus monosymmetros* is described. An embryological explanation of the findings in the heart is attempted. A study of the entire monster suggests that duplication has commenced prior to or at the time of the appearance of the primitive streak and that the state of the heart is due rather to a failure of fusion of the early cardiac rudiments than to a genuine duplication of structures.

I wish to thank Professor Low for placing this specimen at my disposal and for his advice and critical interest in the work. I am also indebted to Mr. G. L. Purser for many valuable suggestions, to Dr. Middleton Cannon for the radiograms, and to Mr. James Moir of this department who prepared the photographs.

LITERATURE CITED

- ETERNOD, A. C. F. 1909 *L'oeuf Humain*. Geneva, p. 79.
FRAZER, E. 1931 *Manual of Embryology*. London.
KEITH, A. 1933 *Human Embryology and Morphology*. London.
SCHWALBE, E. 1906 *Die Morphologie der Missbildungen*. Jena, p. 220.

BRANCHES OF THE AORTIC ARCH IN 153 RHESUS MONKEYS (SECOND SERIES)

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FOUR FIGURES

A second series of aortic arches from macaques is here reported, all of the 153 specimens being from rhesus monkey (*Pethacus rhesus*—Elliot, '13, or *Macaca mulatta*—Miller, '33). In the first series of arches from 133 macaques (De Garis, '35) 115 specimens were from *P. rhesus*, the remainder being from *sinicus*, *fascicularis*, *irus*, *mordax* and *nemestrinus*. Questions of terminology regarding these species have been dealt with in the 1935 report.

The animals from which aortic arches of the present report were taken have been supplied from the Carnegie colony, Baltimore, supervised by Dr. C. G. Hartman and from New Haven through the courtesy of Prof. J. F. Fulton. Specimens from these sources were kindly transmitted by Dr. A. H. Schultz, recorder of the Hopkins anatomical collection. Large additions were also made by Dr. H. A. Howe, these being available in the course of his work on poliomyelitis at the Johns Hopkins Medical School through the Roosevelt Warm Springs Fund. To all of the above contributors my thanks are due.

The series of aortic patterns described here is regarded as large enough for significant statistical treatment, especially since all specimens are from a single species. These patterns are classified on the basis of criteria established in the previous report, and as in that report their incidence is represented by polygons of frequency.

PATTERNS AND INCIDENCE

In the previous report an attempt was made to treat statistically the twenty-two aortic specimens from macaque reported by Keith (1895) and the nine reported by Parsons ('02). Those excellently described and figured by Rojecki (1889) were not included, since his varieties of aortic patterns were not cited in reference to species or numbers of specimens. In the present report no further account is taken of specimens from the literature. All the materials described here belong to an original series, in no part taken from the work of other authors nor in any wise previously reported.

The patterns of aortic branches in rhesus monkey are here figured in their order of frequency as reported in man (De Garis, Black and Riemenschneider, '33), i.e., the classification shown in figure 1 starts with the normal human sequence of three separate trunks and passes through stages of cephalad migration of the *a. carotis communis sinistra* upon the *anonyma* until the typical mammalian type of the long *truncus communis* is reached. In all of these patterns the *a. subclavia sinistra* is a separate aortic stem, its distance from the *truncus communis* apparently bearing no relation to the length of this *truncus* or to other diverse arrangements of aortic stems.

Below are given brief descriptions of the various aortic patterns found in the materials examined (fig. 1); these, it should be emphasized, are from the single species, *P. rhesus*. Also notation is made of the numbers of specimens showing each pattern and the percentage incidence of such pattern, both in report I ('35) and in the present report, hereafter referred to as report II. For further statistical treatment are shown the percentage difference (P diff.) of each pattern in the two reports and the standard error of the difference (E diff.).¹ Only when the percentage difference is greater

¹ The mean or standard error E_a , which follows any given percentage A , is computed from the formula $E_a = \sqrt{\frac{A \times (100 - A)}{n}}$ where n is the total number of specimens. The percentage difference P diff. is simply the difference between any two percentages; the standard error of the difference E diff. is computed from the formula $E \text{ diff.} = \sqrt{E_a^2 + E_{a_i}^2}$ where E_a is the standard error of one percentage, E_{a_i} the standard error of the other percentage. (See Fortuyn, '29.)

than three times the standard error of the difference does it become likely that such a difference is statistically significant. In much of the literature on variation the probable error is employed; this is 0.6745 times the standard error and deviations as great as four times the probable error may be due to chance. The percentage incidence, as noted below, is the basis on which polygons of frequency are constructed.

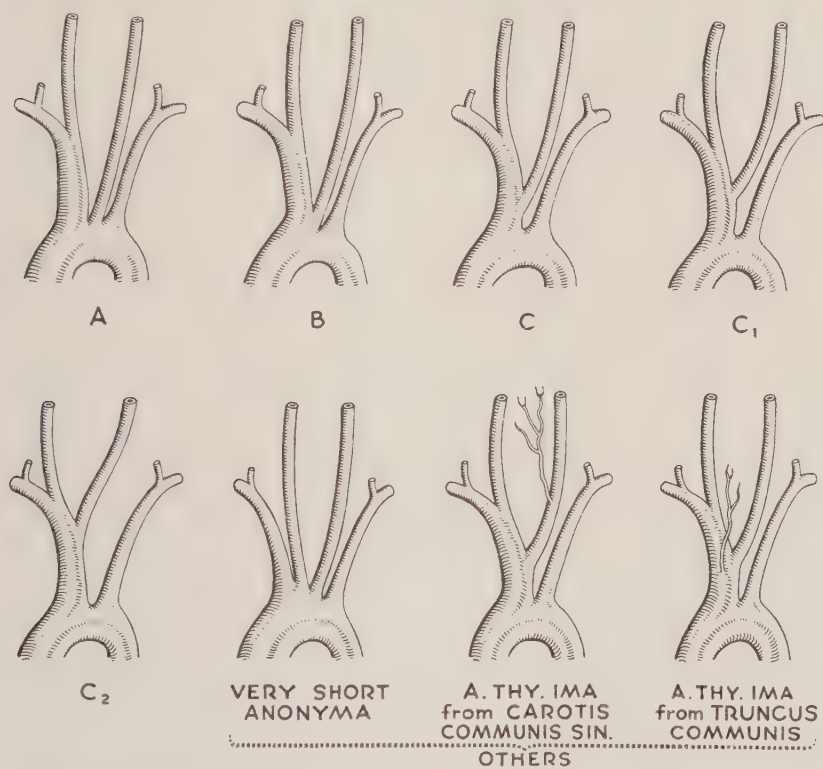


Fig.1 Diagrams of aortic patterns in rhesus monkey: A, the usual human sequence of three separate trunks from right to left as follows: 1) a. anonyma, 2) a. carotis communis sinistra, 3) a. subclavia sinistra; B, radix communis for anonyma and carotis communis sinistra, a frequent arrangement in man; C, short truncus communis for anonyma and carotis communis sinistra, also frequent in man; C₁, truncus communis proportionately intermediate in length between that in man and that usually found in mammals; C₂, long truncus communis usually found in mammals. Others, being three patterns not falling in the above categories: 1) an extremely short anonyma, the arrangement closely approaching that of four separate trunks, 2) the a. thyroidea ima arising from the carotis communis sinistra, 3) the same artery arising from the truncus communis.

Pattern A. This pattern represents the usual human sequence of aortic stems, i.e., from right to left: 1) anonyma, 2) carotis communis sinistra, 3) subclavia sinistra. This arrangement was found in fourteen specimens of report I ($12.17 \pm 3.04\%$) and in thirteen specimens of report II ($8.49 \pm 2.2\%$). P diff. 3.68, E diff. ± 3.75 .

Pattern B. This is a frequent variant of the human aorta and is peculiar in that the anonyma and carotis communis sinistra arise by a radix communis, i.e., the angle made by the anonyma and the convexity of the aortic arch is occupied by the carotis sinistra. To test this arrangement as a distinctive pattern it is necessary to open the aortic arch along its concavity and turn it inside out, thus demonstrating whether the anonyma and carotis sinistra have a common exit from the aorta. This pattern was found in fifteen specimens of report I ($13.04 \pm 3.13\%$) and in seventeen specimens of report II ($11.11 \pm 2.53\%$). P diff. 1.93, E diff. ± 4.02

Pattern C. This pattern represents a stage in progression toward the prevailing mammalian arrangement of aortic stems, and is frequently found in man. It consists of two aortic branches, the first the truncus communis for anonyma and carotis sinistra, the second the subclavia sinistra. In this and the previous series of macaques the truncus communis varied greatly in length, only the short truncus being regarded as comparable to that in man.

Definitions of short, intermediate and long common trunks have been set forth in report I, with the reasoning and computation on which these definitions rest. The short truncus communis in macaque is taken to be any trunk with a maximum length of 6 mm.

Pattern C was found in sixty-two specimens of report I ($53.91 \pm 4.68\%$) and in eighty-nine specimens of report II ($58.17 \pm 3.98\%$). P diff. 4.26, E diff. ± 6.14 .

Pattern C₁. This pattern is characterized by an intermediate truncus communis, i.e., one more than 6 mm. and not over 11 mm. long. It was found in twelve specimens of report I ($10.43 \pm 2.84\%$) and in twenty-five specimens of report II ($16.33 \pm 2.98\%$). P diff. 5.9, E diff. ± 4.11 .

Pattern C₂. This, the prevailing mammalian arrangement of aortic stems, has a long truncus communis, i.e., one of any length beyond 11 mm. A single specimen of the present series had a truncus communis 27 mm. long, an excessive length for macaque. Pattern C₂ was found in nine specimens of report I ($7.82 \pm 2.5\%$) and in six specimens of report II ($3.92 \pm 1.56\%$). P diff. 3.9, E diff. ± 2.94 .

Other patterns. Three specimens of the present series, accounted in figure 1 as 'others,' do not properly fall within any of the above categories. One specimen is remarkable in that the anonyma is extremely short (scarcely 1 mm.), thus closely approaching the condition of four separate aortic stems in the sequence: subclavia dextra, carotis com. dextra, carotis com. sinistra, subclavia sinistra. This sequence is very rare in man and apparently has not been found at all in other primates. Tiedemann (1822) records nine human cases from the European literature, but in the large human series of Thompson (1893) and Adachi ('28) no mention is made of such a pattern. Of interest in this connection is a conspicuous error in the report by Vallois ('29), repeated by Weinert ('32).

The remaining two specimens exemplify the a. thyreoidea ima. In one of the specimens this artery arises from the carotis sinistra and is distributed to the esophagus, trachea and glandula thyreoidea. In the other specimen it arises from the truncus communis for anonyma and carotis sinistra 8 mm. from the aortic arch and is distributed in part to the thymus; the larger part of its distribution is not available. Patterns of the aortic arch involving the a. thyreoidea ima are apparently the rule rather than the exception in chimpanzee; of ten specimens reported by Glidden and De Garis ('36) eight had this artery arising either from the arch or from the root of the carotis sinistra. This artery as a branch of the carotis sinistra has been recorded by Keith (1895) in two macaques.

Patterns designated as 'others' occurred in three specimens of report I ($2.6 \pm 1.48\%$) and in three specimens of report II ($1.96 \pm 1.25\%$). P diff. 0.64, E diff. ± 1.93 .

SUMMARY OF INCIDENCE

The incidence of the foregoing aortic patterns in rhesus monkey is summarized by polygons of frequency (fig. 2). It is seen from these polygons that the pattern of the short truncus communis (C), which is within a maximum length of 6 mm., is the norm from which variations must be computed.

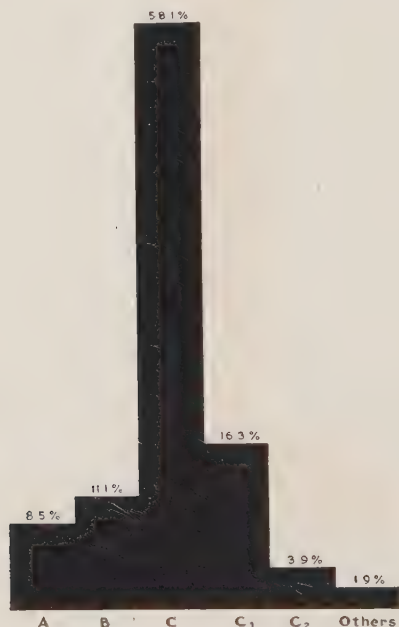


Fig. 2 Polygons showing percentage frequency of aortic patterns in the present series of 153 specimens of rhesus monkey. The pattern designations (A, B, C, etc.) are those of figure 1.

On the basis of 153 specimens it is found that eighty-nine, or $58.17 \pm 3.98\%$, fall inside this norm. It is further seen that variants to the left of the norm are those with distinctly human patterns (A and B) and comprise a total of thirty specimens ($19.6 \pm 3.2\%$), while variants to the right of the norm are those with prevailing mammalian patterns (C₁ and C₂), or with aberrant patterns of various kinds ('others'), the total to the right being thirty-four specimens ($22.22 \pm$

3.36%). The total number of variants, i.e., the sum of those on both sides of the norm, is sixty-four (41.8 ± 3.99). Excluding aberrant patterns, there are almost equal numbers (30 and 31) of specimens in the two general categories, mammalian and human, respectively on right and left sides of the



Fig. 3 The solid black contour is that of figure 2. The contour superimposed within broken lines (black on white or white on black) defines the polygons of percentage frequency for aortic patterns in the series of report I (115 specimens of rhesus monkey). The original polygons illustrating report I were based on absolute numbers of specimens per pattern, not on percentage relations.

norm, which latter then occupies an intermediate position between the above two categories and shows a very strong modal value in this position.

A direct comparison between incidence of aortic patterns in the present report (II) and that of the same patterns in report I is shown in figure 3, where polygons of percentage frequency in the two reports are superimposed. A statement in report I is of interest in this connection (p. 157):

With a series as large as this (115), comprising samples of a single species, it is possible to draw certain fairly definite conclusions:—that for *P. rhesus* the norm of aortic branches is a truncus communis within the range of 6 mm. maximum length; that this norm is within the range of comparable length for the human truncus communis; that this norm stands intermediate between the distinctly human patterns on one side and the distinctly mammalian patterns

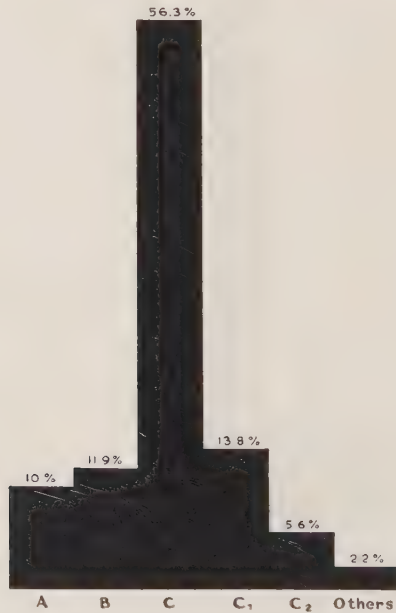


Fig.4 Polygons showing percentage frequency of aortic patterns in the combined series of 268 specimens of rhesus monkey (115 of report I and 153 of report II).

on the other; that variations are almost equally distributed about the norm. Given another large series of the same species one would expect all the foregoing conclusions to hold

It is readily apparent from figure 3 that for the present series of 153 specimens all of the above conclusions do hold. That a high degree of mathematical congruence would be found between two such sets of frequency polygons was predicted in report I, provided: 1) all specimens were from the

same species, and 2) were random samples. These two conditions have been fully met in the present series. The comparison of incidence for each pattern is set forth in the descriptive text above by means of the percentage difference (P diff.) and the standard error of the difference (E diff.). It is seen that for each pattern the difference between the two percentages (reports I and II) is much less than three times its standard error, from which circumstance it may be said that the difference shown in the polygons is in each case simply a matter of chance.

The percentage frequency of aortic patterns in the combined series of 268 specimens of rhesus monkey (those of reports I and II) is shown in the polygons of figure 4. These polygons are of course statistically closer to an expression of prevailing incidence than are the polygons of figures 2 and 3, since they are based on a much larger series. Yet by the same token such differences as there are have been proportionately reduced. It may be predicted here, as in report I, that given another series as large as this, the polygons of frequency would show a high degree of mathematical congruence, provided: 1) all specimens were from the same species, and 2) were random samples, i.e., not in any way selected.

DISCUSSION

The importance of dealing with a single species and the results obtained by introducing materials from other species have been clearly demonstrated in report I, and require no further discussion here. The insistence on material which is not in any way special, i.e., not selected as to source or pedigree, is a matter of much importance in the light of work reported by Edmonds and Sawin ('35, '36), who found that in 1900 rabbits of known pedigree the usual pattern of aortic branching and variants therefrom showed segregation ratios consonant with a difactorial mendelian inheritance.

An opinion has at times been expressed that the degree of so-called fusion between aortic stems is in some way related to thorax capacity, or in general to certain trunk measurements. This opinion has not as yet been put to statistical test,

for which reason it must remain merely suggestive. However, if it should prove to be true that a close correlation exists between patterns of aortic branching and trunk measurements, further if aortic patterns are shown to be inherited in a given way, then the problem becomes essentially one of finding out whether or not particular types of trunk or sizes of thorax are the basic inherited diversity upon which differences of aortic patterns depend.

The question of bilateral symmetry, as exemplified in the mammalian aortic arch, has been studied by Lewis ('23),² with special reference to the developmental sequence in sheep. He finds that in this mammal the aortic arches pass from their early embryonic symmetry through various asymmetrical arrangements to reach the definitive condition of a single aortic stem. From this stem arise first the subclavia sinistra, then after a short interval the subclavia dextra and a long bicarotid, which latter divides symmetrically into the aa. carotides communes. Thus, except for slight displacement in origin of the subclavian branches, the aortic arch in sheep is restored to a remarkable degree of symmetry.

Lewis cites other mammalian aortic arches, showing other types of symmetry, e.g., the bi-innominate arrangement in certain insectivores, the four-trunk pattern as a variant in the marsupial bear and in some cetaceans, and the bicarotid as an aortic stem independent of the subclavian branches—this latter arrangement found in the Tasmanian marsupial wolf and once by Cuvier in the elephant. Most mammalian aortic patterns, however, are said to be distinctly asymmetrical, many falling within the range of branchings shown above for rhesus monkey. Such patterns Lewis regards as more primitive, in the sense of showing less concentration of right and left aortic stems, or less approach to one of the other types of symmetry. By this criterion the normal human aortic branching would be even more primitive than

² This interesting treatment of the aortic arch was only recently called to my attention by Prof. E. A. Boyden, through whose kindness I was able to examine the reprint.

that found in most mammals, since the truncus communis for anonyma and carotis sinistra, i.e., the usual mammalian pattern, would represent a stage in advance toward symmetry. This view is of special interest to us, since we came to a similar conclusion by different and, as it seems to us now, less complete evidence and reasoning (De Garis et al., '33, p. 616).

The aortic arch is thus seen to be worthy of attention, because its branches offer such a wide range of difference in their arrangement; it is, indeed, a structure peculiarly suited to studies of variation, inheritance and symmetry. The problems involved in these studies seem now at least clearly defined.

CONCLUSIONS AND SUMMARY

Patterns of branching of the aortic arch in a series of 153 rhesus monkeys (*Pithecus rhesus*) show the normal human sequence in thirteen specimens, the radix communis for anonyma and carotis communis sinistra in seventeen, the short truncus communis (maximum length 6 mm.) for anonyma and carotis sinistra in eighty-nine, the intermediate truncus communis (maximum 11 mm.) in twenty-five, the prevailing mammalian type of long truncus six. Variants from the above categories are: 1) an extremely short anonyma, this pattern approaching one of four separate trunks; 2) the a. thyreoidea ima arising from the carotis communis sinistra; 3) the same artery arising from the truncus communis.

The percentage incidence of aortic patterns is expressed in polygons of frequency, and superimposed polygons show frequency of the same patterns in this series and in a previous series of 115 specimens. Pattern frequency in the combined series of 268 specimens is likewise figured. From these polygons it is concluded that the norm is a short truncus communis comparable to that often found in man, and that this norm has a strong modal value intermediate between human and mammalian patterns, with almost equal distribution

of these patterns on either side of the norm. The possible relation of aortic patterns to trunk measurements, the problem of inheritance involved in this question, and the significance of aortic patterns for the general problem of symmetry are discussed.

LITERATURE CITED

- ADACHI, B. 1928 *Das Arteriensystem der Japaner*. Bd. 1. Kyoto.
- DE GARIS, C. F. 1935 Patterns of the aortic arch in a series of 133 macaques. *J. Anat.*, vol. 70, pp. 149-158.
- DE GARIS, C. F., I. H. BLACK AND E. A. RIEMENSCHNEIDER 1933 Patterns of the aortic arch in American white and negro stocks, with comparative notes on certain other mammals. *J. Anat.*, vol. 67, pp. 599-619.
- ELLIOT, D. G. 1913 *A Review of the Primates*. Monograph I, Am. Mus. Nat. Hist., New York.
- EDMONDS, H. W., AND P. B. SAWIN 1935 Variations of the branches of the aortic arch in rabbits. *Reeds. Genetics Soc. of Am.*, pp. 65-66. *Am. Nat.*, vol. 70.
- FORTUYN, A. B. D. 1929 How to compute a percentage and an average. *China Med. J.*, vol. 43, pp. 31-41.
- GLIDDEN, E. M., AND C. F. DE GARIS 1936 Arteries of the chimpanzee (*Pan spec?*). *Am. J. Anat.*, vol. 58, pp. 501-527.
- KEITH, A. 1895 The modes of origin of the carotid and subclavian arteries from the arch of the aorta in some of the higher primates. *J. Anat. and Physiol.*, vol. 29, pp. 453-458.
- LEWIS, F. T. 1923 A note on symmetry as a factor in the evolution of plants and animals. *Am. Nat.*, vol. 57, pp. 5-41.
- MILLER, G. S., JR. 1933 The groups and names of macaques. Chapter I, *The Anatomy of the Rhesus Monkey*. Baltimore.
- PARSONS, F. G. 1902 On the arrangement of the branches of the mammalian aortic arch. *J. Anat. and Physiol.*, vol. 36, pp. 389-399.
- ROJECKI, F. 1889 *Sur la circulation artérielle chez le Macacus cynomolgus et le Macacus sinicus*. *J. de l'Anat. et de la Physiol.*, Ann. 25, pp. 513-561.
- THOMPSON, A. 1893 Second Annual Report of Committee on Collective Investigation of the Anatomical Society of Great Britain and Ireland for 1890-1891. *J. Anat. and Physiol.*, vol. 27, pp. 183-194.
- TIEDEMANN, FR. 1822 *Tabulae Arteriarum Corporis Humani*. Carlsruhe.
- VALLOIS, H. V. 1929 Les preuves anatomique de l'origine monophylétique de l'homme. *L'Anthropologie*, T. 39, pp. 77-101.
- WEINERT, H. 1932 *Ursprung der Menschheit*. 308 S. Stuttgart.

THE PROTOPLASMIC FILMS OF THE FAT CELL, THE WALL OF THE PULMONARY ALVEOLUS, AND THE RENAL GLOMERULUS

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TWO PLATES (SIXTEEN FIGURES)

The presence in the body of films of protoplasm so thin that they cannot ordinarily be seen with the aid of the highest available powers of the microscope has, perhaps naturally, been the subject of doubt by anatomists, many of whom deny the existence of a layer they are unable to demonstrate and build their conception of an organ on the firm base of microscopic visibility. Yet for the physiological chemist, not limited in his investigations to histological methods, no such inhibition exists. By several physico-chemical methods, but chiefly through diffraction by x-ray, the thickness of protein surface films has recently been intensively investigated, and the results have been assembled by Cohn. The depth of proteins spread in monolayers varies, according to different authors, from 10 to 18 $\text{\AA}$. This symbol is used to designate the Angström unit, which is equal to $0.0001\ \mu$. With visible light, objects measuring 2000 $\text{\AA}$ are about the smallest that can be seen. The range of measurements of protein films which are beyond microscopic visibility is thus very wide, and one is not justified in denying the presence of such a film merely because it cannot be seen through the microscope.

Several authors have accepted this view. Bensley and Bensley seek to show the presence of the endothelium of the renal glomerular capillaries, ordinarily invisible in sections except near the nuclei, by the Kallitype method. Lewis,

Policard, Dogliotti and Amprino, and others have recognized that a cell in tissue culture may expand enormously over the surface of the cover glass, because of diminished surface tension, in a film so thin that mitochondria are absent, so thin that it is almost inappreciable when observed on surface view with a clear background and quite invisible in profile view. Such films are found in cultures of various cell types and therefore are not characteristic of any germ layer, but merely indicate the action of protoplasm in general when of the proper consistency and submitted to the proper surface tension. Being almost inappreciable when spread on clear glass, they must obviously become invisible in combination with other tissues, unless treated by special methods. To prove the existence of these thin films by showing them as visible layers is the purpose of this paper.

One tissue of the body in which an invisible film is generally given recognition is fat. The common fat cell of adipose tissue, as described by Schaffer, consists of the fat droplet surrounded by the cell wall, the latter divisible into a thin inner protoplasmic layer enclosing the nucleus at one point of the circumference, and an outer fibrillar membrane containing reticular fibers. He points out that the thickness of the outer membrane depends upon the amount of pressure that may be exerted on the cells; it is very prominent in the plantar adipose tissue and in other regions where the tissue acts as a cushion, and may be thin or even absent where individual fat cells are supported by others, or by connective tissue, or where there is no pressure. In an individual cell the reticular membrane may be present in some portions of the circumference, absent in others. Such a fat cell is shown in part in figure 1, drawn from a section of a human parathyroid gland in which individual fat cells were scattered. The fluid fat has been removed as usual in the preparation of the section, but the nature of the cell is identified by the character of its flattened nucleus, which shows the rounded indentation of a minute fat droplet on the peripheral surface as described by Plenck. Where the cell rim borders on tissue spaces a delicate

membrane is present, but when it rests on the chief cell of the gland and also over part of the capillary surfaces no rim is visible. In the relation of the fat cell wall to the adjacent capillaries this drawing resembles closely many similar drawings of the wall of the pulmonary alveolus, yet, though the pulmonary capillaries are often described as 'naked,' in this fat cell a covering protoplasmic film is readily assumed to exist during life, for without it the fluid fat would escape into the tissue spaces as is often seen in fresh tissue roughly handled, when the cell wall is torn. Actually the film over the capillary is as invisible in the one case as in the other.

Two methods are now available which in combination can demonstrate the existence of this thin protoplasmic layer between the fat cell membrane and the contained fluid fat. One depends on the ability of certain cells to store vital dyes. Within the protoplasm the dye may be in the form of dust-like particles, of small discrete granules or of larger masses, depending apparently on the type of cell involved and the duration of exposure to the dye. In his original paper on the vital dyes Goldmann noticed that adipose tissue was lightly stained, but from his figure it is doubtful whether he distinguished between the actual fat cells and the accompanying histiocytes which take up the dye avidly. Dogliotti and also Clara ('30) both show the presence of granules of the dye in fat cells not only in the thicker visible area near the nucleus, but also at other points of the circumference where the protoplasmic rim cannot ordinarily be detected. In starved fat cells where the protoplasm is plainly visible the granules lie definitely within it. Both these authors consider the presence of the vital dye granules as proof of the existence of the thin protoplasmic rim around the fat globule, even though the latter may be microscopically invisible. The visible granules locally expand the protoplasm in which they lie, while between them the protoplasmic sheet remains thin, impossible to detect.

Only a few of the fat cells in any given area are stimulated to store the vital dye. Dogliotti found as a corollary to this that, when the vital stain is taken up in large amounts by fat

cells which have been deprived of their fat by starvation, then their ability to absorb fat again on refeeding is greatly hindered or entirely suppressed. By the presence of the dye the fat-absorbing functions are 'blocked.' Goldner similarly thinks of the differences of the size of the dye granules in individual fat cells as due not to different phases of cell activity, but to greater or less 'blocking' by the enclosed fat. One might think that the unstained cells of a fat lobule were those completely 'blocked' in this way, a view supported by Dogliotti's finding that in the subcutaneous tissue of drastically starved rats all the fat cells will take up trypan blue. In normal adipose tissue, however, whatever may be the reason, the vital dye is found in most or all of the histiocytes present but in only a few of the fat cells. Even these few react only after massive doses of the dye.

A portion of the rim of such a fat cell is shown in figure 2. A histiocyte loaded with dye granules lies between two fat cells. That on the left has a sloping wall, without granules. That on the right, seen in true profile, contains the trypan blue granules in the protoplasmic rim near the nucleus, and in one direction the granules continue further than the visible protoplasm, looking like a row of unsupported minute beads. In other sectors of the cell, beyond the portion drawn, the rim appears as a single line, probably representing the outer fibrillar membrane only.

In order to locate more definitely the position of the dye granules and to demonstrate clearly that their presence indicates the existence of a protoplasmic layer between the fat globule and the fibrillar membrane, the method recently described by Wassermann was applied to the problem. This author, relying on the physical phenomenon that protoplasm will imbibe fluid when placed in a hypotonic solution, immersed thin layers of adipose tissue for periods of $\frac{1}{2}$ hour or longer in distilled water or in a saturated aqueous solution of thymol. He found a great increase in the weight of the specimens, even up to 100% or more. The swelling is partly in the intercellular substance, partly in the fat cells. In the

latter it is located mostly in the protoplasmic layer, as shown by the facts that the unaltered reticular membrane is definitely recognizable, that each fat droplet is apparently of the same size as in the untreated tissue, though now globular instead of polygonal, the usual shape assumed as the result of mutual pressure, and that between membrane and fat there is a large area in which particles can be seen in brownian movement. This area is the protoplasmic rim of the fat cell. From almost invisible thinness it has swollen by the imbibition of fluid to a thickness of perhaps one-tenth of the diameter of the fat globule, according to this author's photographs. The reaction is reversible, as he also shows clearly, for on immersing a previously swollen piece of adipose tissue in some hypertonic solution or even in normal Ringer's solution for $\frac{1}{2}$ hour the protoplasmic rims shrink to invisibility and the fat globules reassume their polygonal shapes.

Wassermann's technique is easily copied. When carried out on adipose tissue taken from a series of white rats already subjected to massive doses of trypan blue and known (through later examination of sections) to contain the blue granules in occasional fat cells, the location of the dye granules within the protoplasm of the rim is definitely assured. They lie at varying depths from the surface of the cell, mingle with the previously seen protoplasmic granules and, with the latter, exhibit violent molecular movement. Moreover, on the addition to the fluid in which the specimen lies of some hypertonic solution (the reaction can be followed through the microscope), the protoplasmic rim can be seen to shrink to invisibility while the blue granules cease their movement and assume an irregular row around the now polygonal fat globule.

As a variation of this technique the adipose tissue may be placed in a saturated aqueous solution of thionin for 3 to 5 minutes before being immersed in the thymol water. This stain is only very slightly toxic and does not seem to affect the swelling of the protoplasm. The fat cell nuclei are made visible, and are found to be oval or even spherical as they lie in the swollen cytoplasm, since they are no longer compressed between fat globule and fibrillar membrane.

By gently teasing the adipose tissue it is occasionally possible to detach individual cells. Two of these are shown in figures 10 and 11. One has retained its membrane intact, and the protoplasm lying between membrane and fat droplet is marked by protoplasmic granules; the nucleus has been stained with thionin, and is broadly oval. In the other cell, containing the dye granules, the membrane has apparently been ruptured, leaving only the swollen protoplasm. The outline of the cell is in this case practically determined by the position of the granules, since the fluid protoplasm is barely visible. The nucleus has not been stained. On the addition of a drop of acetic acid to this specimen the dye granules were seen to assume a single irregular row about the fat globule, much like that shown in figure 2.

The finer structure of the alveolar wall in the mammalian lung is a present subject of controversy as it has been for several decades. In lower forms, such as the frog, the lung is lined throughout by entodermal epithelium, and in the opinion of some investigators a complete continuous epithelium also exists in the mammalian lung, either in the form of a delicate sheet of squamous cells or as Kölliker's 'respiratory epithelium,' consisting of rounded or cuboidal nucleated cells lying in the meshes of the capillary net and of extremely thin plates or films of protoplasm stretched over the capillaries. The plates have been regarded either as separate non-nucleated cells or as flat extensions or flanges of the cuboidal cells. Others maintain that no continuous epithelium is present, the cuboidal cells being separated by wide gaps through which capillaries come to the surface as naked endothelium or covered by a sort of basement membrane. Still others regard the alveoli and all the thin-walled air cavities as tissue spaces, without a definite epithelial lining of any kind; the few cells seen at the surface are considered mesodermal macrophages. The discussion of the various views on the subject is reviewed in a former paper (Bremer, '35) and need not be repeated here; suffice it to say that as yet the presence of a protoplasmic layer covering the capillaries in

the mammalian lung has not been demonstrated to the satisfaction of all investigators.

It has already been shown that in vitally stained fat cells the presence of a row of dye granules in sections of fixed material may be taken as proof of the existence of an invisible thin film of protoplasm enclosing them, which can be made visible by causing it to swell. Prior to the publication of Wassermann's paper we had noticed a similar arrangement of blue granules at the surface of the alveolar wall of the lung, after what may be called chronic vital staining. When the new technique became available it merely remained to show that the granules have the same significance in the lung as in the fat cell, in that they are contained in a film of protoplasm which can be made visible.

Several authors, among them Francescon, Seemann, Tschistowitsch, and Dogliotti and Amprino, have investigated the lung by the method of vital staining. They are mostly interested in the problem of the relation of the alveolar phagocyte to the 'septal cell,' with which we are not at present concerned; often the dye was administered by the trachea, with results not comparable with those obtained by true vital staining produced by subcutaneous injection. It is generally agreed, however, that although the histiocytes and alveolar phagocytes respond by taking up the dye in large quantities and in large granules, the alveolar epithelial cells or small round cells or septal cells (whatever the individual authors prefer to name them) store the dye only sparingly, and then in the form of small granules. Definite statements as to the relations of these lightly stained cells to the alveolar capillaries are lacking, except that Gazayerli notes that "the flattened lining cells contained no dye." The other authors mostly consider the capillaries 'naked.' Dogliotti and Amprino, who leave this latter question open, seem to show in their figure 4 a similar row of granules covering a capillary in the alveolar wall, but do not call attention to this relation. Weslaw recognizes the entodermal epithelial cells by special staining of their lipoid granules, but does not find these granules in the

thin plates of lower animals and does not see similar plates in mammals.

Two series of white rats were subjected to fourteen doses of trypan blue (1.5 cc. of $\frac{1}{2}\%$ solution) injected subcutaneously on alternate days, and then kept for a resting period without further injections. The animals were killed at intervals of a few days; the lungs were fixed in Susa, sectioned in paraffin and stained with paracarmine and orange G to contrast well with the trypan blue. Storage of the dye in the surface protoplasmic films was first recognized after 12 days of rest (40 days after the first injection), was most marked during the next 4 weeks, and then gradually diminished as elimination exceeded storage. I am greatly indebted to Prof. H. L. Weatherford for bringing to my notice the advantages of this chronic vital staining, and also for many helpful suggestions as this work developed.

As in the case of the fat cells, only an occasional epithelial cell shows the reaction. While apparently every histiocyte is loaded with the dye, many alveoli in sections show no granules at the surface. The areas covered by the closely massed dye particles can be followed in thin serial sections, and reconstructions show that they occupy only a small part of the alveolar surface. Whether each such area of storage represents a single cell or a group of cells is not easy to determine, but in many instances, as is shown in figures 4 and 5, the narrow line of surface granules is continuous with a single mass of perinuclear protoplasm, also storing the dye, and the arrangement of nucleated cuboidal cell body and appended thin expanded film is obvious. No break between cell body and film can be recognized, and in any one case the size of the granules is approximately the same in both positions. The alveolar cell has expanded over the underlying structures in the same way as the cell in tissue cultures expands along the surface of the cover glass.

Why a few only of the alveolar cells store the dye is not clear. No readily supposed cause, such as the suggested 'blocking' in the case of the fat cell, can be invoked. The

same selective action, noted also by other authors, is seen in the cuboidal epithelium of the bronchioles as is shown in figure 6, where only two cells of the epithelial sheet have taken the dye. Nor is it clear why the size of the granules varies in the different cells which do store the dye. Such variations exist, and may help to explain the cellular relations. In figure 3, for instance, the triangular area at the lower part of the larger cell body, marked by a definite grouping of the dye granules, is found to belong to another cell whose nucleus lies in the next section, while the different patterns of the two halves of the thin surface layer may indicate its relation with the two contiguous cells.

From sections of fixed material alone it would be perhaps difficult to determine definitely the position of these rows of granules. Where they cover the alveolar capillaries they might be within the endothelium, for Seemann reports that after intensive vital staining this endothelium will show a few fine granules. In my specimens these single endothelial granules are far apart, and occur on all sides of the vessel, not massed on the wall facing the alveolar surface. Moreover, as is shown in the drawings, the rows of granules are also continuous over connective tissue (fig. 3) and over the bands of smooth muscle found at the mouths of the alveoli (fig. 4). Other single granules of the dye can be found in the tissue spaces throughout the lung, but these could not be confounded with the rows at the surface. The alveolar phagocytes, which might spread out over the surface, can be distinguished by the large size of their granules, which most authors acknowledge to be characteristic. Though their tendency is to become round and float free in the alveolar cavities, they may occasionally send out pseudopodia, but the extensions, as figured by Loreti and Zaietta, are short and thick as compared with those of the epithelium. Finally the dye granules may be merely adherent to the surface of the wall. Others, perhaps released by degenerated phagocytes (for there has been no intratracheal injection in these specimens), float singly or in groups in the alveolar cavities, and some of these might

come to rest against the wall. Occasionally the free groups and the intracellular granules can be differentiated, however. In some places the thin surface layer has been separated, probably during fixation, as noted by Orsós, and appears as a crumpled sheet (fig. 4); similar sheets, containing the small dye granules, have been followed in serial sections, running first so as to show the granules as a single line, then turning into the plane of the section so that the granules formed a scattered group in a definite area. Such an arrangement of the granules, though highly improbable as a chance grouping, is quite compatible with the appearance of a dye-laden protoplasmic film bent so as to be seen first in profile and then on surface view.

Another structure which is more readily differentiated from the thin film with its granules is the collagenous layer described by Seemann as covering the capillaries. After protracted vital staining, the trypan blue colors some, but not all, of the collagenous fibers. Even in animals not vitally stained this layer of fibers sometimes shows signs of color due to refraction. This stained layer can be distinguished from the vitally stained alveolar cells by the recognition of its fibrillar nature, by the slatey color of the fibers as contrasted with the brilliant hue of the trypan blue particles, and by the fact that the layer is, of course, not found in a cell body.

The positive placement of the rows of dye granules in a surface film of protoplasm and the demonstration of this film whether or not it contains the dye can be accomplished by the use of a slight modification of Wassermann's method for fat. The lungs of vitally stained animals are placed first in salt solution. Frozen sections are cut and transferred immediately to warm distilled water or saturated thymol solution. An aqueous solution of thionin, to stain the nuclei, may be used for a few minutes after sectioning. The sections are examined in the thymol solution, without clearing. Such specimens begin to show the swelling of the protoplasm in a few minutes, and retain their value for several hours.

In such swollen frozen sections of the lung occasional limited regions may be found in which an alveolar wall is seen in true profile against a clear background, as shown in figures 12 and 13. The most prominent structures are the refractive elastic fibers, which appear dark in this uncleared material. They are a portion of the continuous angular elastic net, which with interspersed collagenous fibers forms the framework of the alveolar walls (compare Amprino and Ceresa). Sometimes they approach close to the surface, sometimes they lie beneath capillaries. The fine reticular membrane surrounding the capillaries is not recognizable. Covering the elastic fibers or the protruding capillaries is a layer of almost clear material in which the protoplasmic granules show molecular movement. The foamy character of this unstained swollen cytoplasm makes it difficult to see any definite edge at the surface, and there is no special exoplasmic membrane as in the fat cell, but its limits can be estimated by the activity of the granules, which in their molecular motion have a shorter excursion in the protoplasmic gel than in the surrounding fluid, and also remain fixed in position. Sections stained with thionin may show nuclei in the thicker portions between capillaries. Very rarely, and only by fortunate chance, the blue dye granules are found mingled with the protoplasmic granules in such favorably oriented cells. They are smaller than those seen in the macrophages floating by, and much less densely packed. They are at different levels in the film, resembling in this respect those found in the swollen fat cell film, and also occupy the perinuclear portions. On the addition of a drop of acetic acid on the slide they can be seen slowly to cease their motion and assume a single irregular row over the elastic membrane or capillaries. The protoplasmic granules in this condition are unnoticeable. In the swollen condition the thickness of the films is irregular, sometimes approaching that found in the fat cell, sometimes much thinner; even short gaps may occur where there are no granules and the film is invisible, though its local continuity can hardly be doubted.

Since the dye granules are mingled with the protoplasmic granules in these surface cells, the presence of the latter alone is sufficient to demonstrate the films. Vital staining is not necessary. Figure 14 is from the lung of a normal cat treated with the thymol solution, and shows relations of elastic membrane, capillaries, and surface film similar to those found in the vitally stained rats, except for the presence of the blue granules.

I wish to emphasize the fact that only occasionally are areas to be found where the surface layer is demonstrable. In the lung, in addition to the short gaps just mentioned, long stretches of alveolar surface seem bounded by naked elastic membrane or by naked capillaries, as seen in figure 14. One explanation of this may be that in these regions the film is so thin that it contains no protoplasmic granules or mitochondria, as Lewis noted in his tissue cultures, and therefore is unrecognizable by the method used, which depends in great part on the presence of these granules. Or it may be a question of orientation; as compared with the large size and even contour of the fat droplet of adipose tissue the small size and intricately woven pattern of the pulmonary capillaries offer much less opportunity for true profile views, undisturbed by refractions in this uncleared material. Various methods of fixation attempted in the hope of securing thin cleared sections have failed to preserve the swollen condition. A complete lining of the alveoli has not been proved; its frequent presence only is demonstrated. The same reasoning may be applied to the endothelium of the pulmonary capillaries, which is not demonstrated as a swollen sheet in these specimens.

One early reference to the epithelial plates of the pulmonary alveoli must be given special recognition here. As I have noted in a former paper on the lung ('35), the second volume of the *Lancet*, published in 1874, contains a letter from Hy. Brown, in which he reported finding the epithelium of the alveoli. His method was to place slices of fresh pig's lung cut as thin as possible with sharp scissors in distilled water

for 15 minutes, then to transfer them to fresh distilled water for a further period and then to a slide with cover glass. The addition of 1 drop of a very weak solution of acetic acid brought out "beautiful epithelial scales with a nucleus." He attributes his success to the weakness of the acid solution, stating that stronger acetic acid, turpentine, glycerine and other materials all fail to bring out the alveolar epithelium. We can now realize that the result was due to the use of distilled water for several minutes; the addition of acetic acid usually causes a shrinkage of the swollen protoplasm, but in such weak solution may merely bring out the nuclei. Nevertheless, Brown should be recognized as perhaps the first to have seen the ordinarily invisible alveolar films.

In the same paper on the lung I called attention to the method of its postnatal growth by means of minute tubular sprouts which burrow into the loose tissue, interlobular, peribronchial or subpleural, and there expand into new alveoli. They are continuations or extensions of the older alveolar epithelium and may take the form either of 'respiratory epithelium' in relation with capillaries or of sheets of cuboidal cells where capillaries are not in contact with them. A sprout of the latter type, which can be traced in the serial sections to a neighboring alveolus, is shown cut obliquely in figure 7. As is the case in the bronchiolar epithelium drawn in figure 6, two only of the epithelial cells are storing the dye granules. The similarity of the reaction in these two regions gives further indication of the relationship of the cells lining the bronchioles, which all acknowledge to be entodermal, with those of the sprouts and therefore of the younger alveoli. If the 'alveolar phagocytes' are derived from the surface cells of the alveoli, they must be entodermal.

The renal glomerulus provides another example where the relation of the capillaries to the free surface, the presence or absence of an intervening basement membrane or film of protoplasm are matters of controversy. One school, including most of the older anatomists and Bensley and Bensley, Zim-

mermann and others among the more recent authors, recognizes a continuous sheet of epithelial cells resting on a structureless basement membrane; another school, typified by Bargmann and Clara, finds as a covering of the capillaries only widely-spaced individual 'Deckzellen' or 'epicytes,' whose spreading processes clasp the endothelium, leaving large areas of it 'naked.' Certain authors would seem to have demonstrated such a covering in adequate drawings, yet others deny its presence. Some of the differences of opinion may be due to the use of different methods; it seems wise to attack the problem from a newer angle.

With regard to the vital staining of this covering there are also differences of opinion. Von Möllendorff found storage of the dye in the endothelium and in the 'Deckzellen' of cold-blooded animals and of the immature mouse, but not in the 'Deckzellen' of adult mammals. Bargmann, while suggesting that in a tissue primarily designed for excretion such storage might not be expected, yet demonstrated the granules in small numbers in the 'Deckzellen' after prolonged action of the dye. Clara ('36), on the other hand, finds no storage in the surface cells except in two cases, one of which he regards as hyperplastic. He also found none in the parietal cells of Bowman's capsule.

In paraffin sections of the kidney from white rats submitted to chronic vital staining instances where the glomerular surface cells have taken up the dye granules are even more rare, or less often readily demonstrable, than in the lung. That they may be found is shown in figures 8 and 9. The granules are scarcer in the body of the cells, and in the prolongation over the capillary they often do not form so continuous a row, being more discrete or in small groups. Some of the single granules might lie in the endothelium which is often seen to contain them in other parts of the capillary wall. Incidentally the flattened epithelial cells of Bowman's capsule, contrary to Clara's report, often show dye granules after this prolonged treatment. The similarity of these pictures to those of the pulmonary alveoli is striking, but the actual placing

of the granules and the demonstration that they imply the presence of a continuous surface film, ordinarily invisible, call for other methods.

The proof is supplied, as in the case of the lung, by the use of the thymol method. Frozen sections of the kidney from vitally stained rats, some treated with thionin, are immersed in thymol solution. Other sections from normal animals, not vitally stained, were used as controls. As the sections thaw, those of the glomeruli not attached by their vascular poles drop to the bottom of the dish, from which they can be recovered by means of dissecting microscope and pipette to form excellent objects for study. In these and in the glomeruli still attached to the sections the surface is occasionally presented in true profile against a clear background, and when so fortunately oriented shows areas in which a continuous sheet of protoplasm is made visible by its granular content (fig. 15). Most of these areas, even in the vitally stained material, contain only the normal protoplasmic granules; rarely the dye granules also are present (fig. 16) indicating the presence of an epithelial cell in the storage stage, or at least one containing granules whether storing or excreting. Both types of granules exhibit molecular movement. The surface layer between the capillaries is deep and often contains round or oval nuclei, while over the capillaries it is of varying thickness.

The dye granules seen in paraffin sections are thus located in the surface protoplasm, and these surface films are found to be much more frequent than study of paraffin sections would suggest, yet in the glomerulus as in the alveolus there still remain certain areas of the surface which in the swollen material show no protoplasmic granules to indicate the presence of a surface film over the capillaries. As in the lung we may suppose these areas to represent films without mitochondria, regions of poor orientation where the true picture is blurred by refraction, or the actual absence of any covering layer.

As was the case with the lung, so in the renal glomerulus, the authors of another paper have previously made use of hypotonic solutions to reveal the thin protoplasmic film. Bensley and Bensley state that their acid citrate solution, which is definitely hypotonic, "swells the protoplasm of the glomerular epithelial cells and makes thin sheets of it apparent over the surface of the glomerular capillaries, where under ordinary conditions of fixation it would be difficult to distinguish it from the basement membrane of the capillary." Their accompanying illustrations are similar to mine, except for the correlation with the chronic vital staining, and for the fact that they are able to show a continuous epithelium throughout the glomerulus. They add a welcome support to my findings.

In both the pulmonary alveolar wall and the renal glomerulus films of protoplasm overlying the capillaries have been demonstrated as continuous sheets for considerable distances where observation is favorable. In the same positions other authors have described discontinuous 'Deckzellen' or epicytes, terms extended from the glomerulus to the alveolus. The discrepancy between these two ideas is tentatively explained by the Bensleys who show a continuous film and postulate that the cytoplasm of the covering epithelial cells is of two densities, and that "it is probably the greater resistance of the denser cytoplasm to extension that gives rise to the ridges and bands mistaken by von Möllendorff and Bargmann for cell processes." The latter two authors admit the possibility of a web between the processes, but do not consider its presence proved. Clara ('36) discusses the problem at length, and maintains that such webs do not exist, or at least are not visible, and that the epithelial layer is discontinuous. Zimmermann agrees with the Bensleys and shows in his drawings that the processes are actually thickened ridges in a continuous cytoplasm, forming a branching pattern of varying design and distinctive staining reactions, connected by veil-like films. He objects to the technique formerly recommended by Bargmann (injection of the kidney after ligation of the renal vein) as leading to the stretching and dilating of the

capillaries and thus altering the normal relations of the overlying epithelium. Yet Clara insists that the epicytes can best be seen in the lung by injecting until the capillary loops have been straightened out.

Forcible stretching of a thin layer is surely not the best method of investigating its existence. In the paraffin sections of vitally stained lungs the dye granules have been seen, but only in one or two cases, to assume rows possibly suggesting the branching of epicytes; in the thymol material they are too scattered to give any such picture. The gaps in the rows of granules seen in profile, noticeable occasionally in both paraffin and frozen sections, may represent the thin webs. Such appearances, however, are so infrequent that the results of this present work may be taken as upholding the ideas of the Bensleys and of Zimmermann that the 'Deckzellen' or epicytes represent only the thicker or visible portions of cells, the real contours of which are smooth and regular.

CONCLUSIONS

In three different tissues of the body the presence of protoplasmic films so thin as to be ordinarily invisible can be demonstrated by their ability to store dye granules after chronic vital staining and to absorb fluid from hypotonic solutions and thus to swell to readily visible dimensions. When these two methods are combined, the dye granules can be found in the swollen films. In the fat cell the ordinarily invisible protoplasmic film lies between the fat droplet and the reticular membrane. In the pulmonary alveoli the films cover the capillaries, apparently as prolongations or flanges of the nucleated surface cells, and are also continuous over the connective tissue bundles or smooth muscle bands when these approach the surface. In the renal glomeruli they similarly cover the capillaries as expansions of the surface cells. In the two latter positions they are thus found where 'Deckzellen' or epicytes have been described, and the presence of the locally continuous films shows that the branching processes of the epicytes are merely the denser portions of

the films which actually are connected by even more delicate webs.

In the lung and the kidney the films cannot be shown to form a continuous sheet; many areas exist where they cannot be demonstrated by these methods. As is the case in the fat cells, only occasional alveolar or glomerular cells store the vital dye. In the swollen material the recognition of the films depends on the presence of granules, either protoplasmic or of the trypan blue. Since films so thin as to contain no mitochondria or granules have been recognized by several authors in tissue culture, it is suggested that these would still be invisible in the swollen material, and that this would account for the apparently 'naked' areas. Actually the presence of protoplasmic films covering the capillaries of the alveolus and of the glomerulus is demonstrated in many areas, but not in all. The probability, however, of a continuous sheet of epithelium in these positions is greatly advanced.

LITERATURE CITED

- AMPRINO, R., AND F. CERESA 1937 Trasformazioni nella struttura del polmone nel periodo postnatale e senile. *Arch. ital. di anat. e di embriol.*, vol. 38, pp. 428-446.
- BARGMANN, W. 1935 Zur vergleichenden Histologie der Lungenalveole. *Zeitsch. f. Zellforsch. u. mikr. Anat.*, Bd. 23, S. 335-360.
- BENSLEY, R. R., AND R. D. BENSLEY 1930 The structure of the renal corpuscle. *Anat. Rec.*, vol. 47, pp. 147-175.
- BREMER, J. L. 1935 Postnatal development of alveoli in the mammalian lung in relation to the problem of the alveolar phagocyte. *Carnegie Contrib. to Embryol.*, vol. 25, pp. 85-111.
- BROWN, HY. 1874 Do the alveoli of the lungs possess squamous epithelium? *Lancet*, vol. 2, p. 681.
- CLARA, M. 1930 Bau und Entwicklung des sogenannten Fettgewebes beim Vogel. *Zeitsch. f. mikr.-anat. Forsch.*, vol. 19, pp. 32-113.
- 1936 Vergleichende Histobiologie des Nierenglomerulus und der Lungenalveole. *Zeitsch. f. mikr.-anat. Forsch.*, vol. 40, pp. 147-280.
- COHN, E. J. 1935 The chemistry of the proteins and amino acids. *Ann. Rev. Biochem.*, vol. 4, pp. 93-148.
- DOGLIOTTI, G. C. 1928 Ricerche sperimentali sulla natura del tessuto adiposo mediante la colorazione vitale con colori acidi. *Zeitsch. f. Zellforsch. u. mikr. Anat.*, Bd. 8, S. 222-248.
- DOGLIOTTI, G. C., AND R. AMPRINO 1932 Ricerche sulla struttura dell'alveolo polmonare. *Arch. ital. di anat. e di embriol.*, vol. 30, pp. 1-16.

- FRANCESCON, A. 1930 Ricerche sulla colorazione vitale del polmone in particolari condizioni sperimentali. Boll. Soc. ital. di biol. speriment., vol. 5, pp. 533-536.
- GAZAYERLI, M. E. 1936 On the nature of the pulmonary alveolar lining and the origin of the alveolar phagocyte. J. Path. and Bact., vol. 43, pp. 357-366.
- ' GOLDMANN, E. 1909 Die äusserer und innere Sekretion des gesunden Organismus in Lichte der vitalen Färbung. Tübingen.
- * GOLDNER, J. 1936 Histiozytäre Abstammung der Fettzellen. Zeitsch. f. Zellforsch. u. mikr. Anat., Bd. 24, S. 312-319.
- LEWIS, W. H. 1923 Amniotic ectoderm in tissue-cultures. Anat. Rec., vol. 26, pp. 97-117.
- LORETI, F., AND A. ZAIETTA 1936 Ricerche sulla natura delle piccole cellule nucleate della parete alveolare e sulla colloidopessia delle cellule polmonari. Lo sperimentale, Arch. di biol., vol. 90, pp. 3-55.
- VON MÖLLENDORFF, W. 1927 Einige Beobachtungen über den Aufbau des Nierenglomerulus. Zeitsch. f. Zellforsch. u. mikr. Anat., Bd. 6, S. 441-450.
- ORSÓS, F. 1933 Das Epithel der Lungenalveolen. Centralbl. f. allg. Path. u. path. Anat., Bd. 57, S. 81-88.
- PLENK, H. 1927 Über argyrophile Fasern (Gitterfasern) und ihre Bildungszellen. Ergebn. d. Anat. u. Entw., Bd. 27, S. 302-412.
- POLICARD, A. 1929 Les nouvelles idées sur la disposition de la surface respiratoire pulmonaire. Press méd., T. 37, pp. 1293-1295.
- SCHAFER, J. 1930 Die Stützgewebe. Handb. d. mikr. Anat. d. Menschen, von Möllendorff, Bd. 2, S. 74.
- SEEMANN, G. 1931 Histobiologie der Lungenalveole. Jena.
- TSCHISTOWITSCH, A. N. 1935 Über die Veränderungen des Lungenparenchyms und Stromas bei der Entzündung. III. Mitteilung: Über die Genese der Alveolarphagozyten. Zeitsch. f. Zellforsch. u. mikr. Anat., Bd. 22, S. 457-466.
- * WASSERMANN, F. 1937 Experimenteller Nachweis der Membran der Fettzelle und ihrer Bedeutung für die Fettorgane als Wasserspeicher. Zeitsch. f. Zellforsch. u. mikr. Anat., Bd. 26, S. 115-145.
- WESLAW, W. 1934 Contribution a l'histophysiologie et l'histopathologie de l'épithélium pulmonaire des vertébrés. Posen.
- ZIMMERMANN, K. W. 1933 Über den Bau des Glomerulus der Säugerniere. Zeitsch. f. mikr.-anat. Forsch., vol. 32, pp. 176-278.

PLATE 1

EXPLANATION OF FIGURES

1 Fat cell in human parathyroid gland. Part of the curved wall is drawn, showing the nucleus with indentation for nuclear fat droplet. The fat cell wall is indistinguishable when in relation with the gland cell (above) and in some places where it meets the capillaries. Paraffin section.

2 Adipose tissue of white rat vitally stained with trypan blue. Parts of two fat cells are shown, one storing dye granules in the protoplasmic rim, the other shelving, without granules. Between them is a histiocyte also loaded with the dye. Compare figures 10 and 11.

3, 4 and 5 Lungs of white rats, chronic vital staining. Alveolar surface toward top of page. Certain surface cells contain dye granules, and are prolonged as films, distinguished as rows of minute granules, which cover capillaries, connective tissue or smooth muscle bundles. In figure 4 the granules are absent in the center of the area drawn, but the film is seen partly detached. Compare figures 12 and 13.

6 and 7 Wall of terminal bronchiole of vitally stained rat, and section of growing tubular sprout in subpleural tissue. In both only two of the cuboidal cells are storing the dye granules.

8 and 9 Renal glomeruli of white rats, chronic vital staining. Bowman's capsule is shown above, the epithelium containing dye granules. Below the glomerular space are the tips of glomerular lobules, showing some of the surface cells with dye granules in the perinuclear cytoplasm and extending in rows over the capillaries, indicating the presence there of protoplasmic films. Compare figures 15 and 16.



PLATE 2

EXPLANATION OF FIGURES

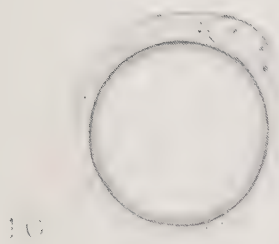
10 and 11 Isolated fat cells from mesenteric adipose tissue of white rat subjected to chronic vital staining with trypan blue. Saturated thymol solution. Figure 10, also stained with thionin, shows nucleus, membrane and protoplasmic rim with protoplasmic granules, but was not storing the dye. In figure 11 the membrane is ruptured, the swollen film contains both protoplasmic and dye granules, some drawn above, not within, the fat droplet. Compare figure 2.

12 and 13 Lung of rat, chronic vital staining. Frozen section in thymol solution. (Fig. 12 also stained with thionin.) Alveolar wall shows protoplasmic film covering elastic tissue and capillaries, the latter seen as empty rounded spaces, since the swollen spherical blood corpuscles are not drawn. Both protoplasmic and dye granules are present in the film and around the nuclei. Compare figures 4 and 5.

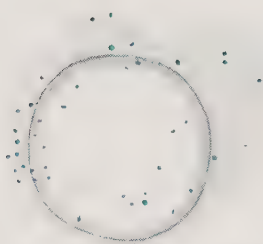
14 Similar frozen section of lung of normal cat; thionin, thymol solution. The walls between three alveoli are shown. The protoplasmic granules which mark the film are absent in certain regions of the two lower alveoli.

15 Kidney of normal cat, frozen section, thionin, thymol solution. At vascular pole of glomerulus the epithelium of Bowman's capsule is continuous with the film over the glomerular capillaries, marked by protoplasmic granules.

16 Kidney of rat, chronic vital staining. Frozen section, thionin, thymol. The tip of a lobule of the glomerulus shows one cell loaded with dye granules and protoplasmic granules, neighboring films with the latter only. Compare figures 9 and 10.



10



11



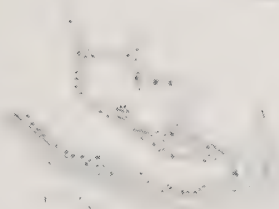
12



13



14



15



16

COMMENTS ON THE ORIGIN AND GROWTH PATTERN OF THYROID PARENCHYMA

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THREE PLATES (TWENTY-ONE FIGURES)

INTRODUCTION

The formation of colloid-filled vesicles from solid cell masses and plates of early thyroid material is difficult to interpret. The developmental patterns of thyroid glands in most cases give no key to the fundamental factors responsible for the adult structure. The thyroid gland of the cat, however, exhibits developmental characteristics which seem to clarify otherwise obscure processes.

To Norris, who has studied the early development of the thyroid gland, including the origin of the follicles in the human ('16 and '18 b) and in *Squalus acanthias* ('17 and '18 a), is due credit for much of the present knowledge concerning the growth pattern of thyroid parenchyma. By the use of graphic and plastic reconstruction methods, Norris has shown that the early thyroid anlagen of the above-mentioned forms become transformed into irregular plates two cells thick from which follicles are later developed. In the early anlagen Norris described the formation of 'intraglandular cavities' which when first seen are tiny clefts between the thyroid cells and later, becoming larger, open at the outside of the epithelial masses to be immediately invaded by the mesenchyme. These cavities bear no relation to the cavity of the thyroglossal duct (or the cavity of the sometimes hollow thyroid diverticulum) and are entirely independent of the later formed

follicular cavities as well. Norris notes intraglandular cavities in the human ('16 and '18 b), in *Squalus acanthias* ('17 and '18 a), and in pig, sheep, dog, rabbit and pigeon ('18 b). Godwin ('36) failed to confirm Norris' observation on their presence in the dog's thyroid. Born (1893) mentioned cavities in the pig's thyroid gland which Norris believes should be placed in this category. Bradway ('29) subsequently reported the presence of intraglandular cavities in the thyroid of the chick.

The writer has seen the intraglandular cavities of Norris in the more massive regions of the cat's thyroid anlage, that is, in its midportion. In addition, beginning with the diverticulum stage in the development of the cat's thyroid and continuing into the adult condition, a very different set of cavities (primitive follicles) is present. It is with the consideration of these that the present paper is primarily concerned. Due to the necessity of distinguishing these cavities from ordinary follicles, a brief description of thyroid folliculogenesis in general must be included.

The entire Cornell collection of cat embryos was used in this study. These embryos are cut serially in the characteristic planes and are stained either en toto with Mayer's HCl carmine or with hematoxylin and eosin. In addition the thyroid regions of a graded series of fetuses, starting at 41 mm. (C. R.) length, were sectioned serially and stained with hematoxylin and eosin.

The major portion of this investigation was conducted in the laboratory of histology and embryology, Cornell University, under the guidance of Prof. B. F. Kingsbury whose kind assistance I wish to gratefully acknowledge. The manuscript was completed at The Daniel Baugh Institute of Anatomy of the Jefferson Medical College. I wish to express my thanks to Prof. J. Parsons Schaeffer for his sympathetic interest and encouragement.

OBSERVATIONS

To facilitate order in presentation, this description of thyroid morphogenesis is separated arbitrarily into five divisions: 1) plate stage, 2) diverticulum formation, 3) cavity formation, 4) prefollicular, and 5) follicular stages.

1. *The plate stage.* The pharyngeal epithelium in the youngest embryo employed (11 somites) gives no indication of a definite area for thyroid formation. The epithelium of the future thyroid plate is similar to that of the pharyngeal lining in general being discernible only by its position and by the notable absence of mesenchymal tissue between it and the underlying aortic sac. The latter characteristic condition persists until the plate becomes transformed into a diverticulum (4 to 5 mm.). While there appears a definite mid-ventral longitudinal groove through the propharynx in a 14-somite embryo, it is not limited to the thyroid area. Embryos of 16 and 18 somites exhibit a slight depression in the pharyngeal floor immediately over the aortic sac and between the ventral grooves of the first pair of pharyngeal (entodermal) pouches. This is the first indication of a delimitation in the thyroid area. Embryos with 22 and 23 somites possess thyroid plates easily recognizable not only by being definitely depressed (thyroid pit) but also, for the first time, by the enormous increase in mitotic figures. Figure 1 illustrates a well-formed thyroid plate (25 somites) in direct contact with the endothelium of the aortic sac. The area shows a characteristic depression (thyroid pit) as well as numerous mitotic figures, and the growth activity is indicated by a local thickening of the plate epithelium.

2. *Diverticulum formation.* The active growth illustrated above (fig. 1) and not the presence of the thyroid pit initiates diverticulum formation. Figures 2 and 3 (4 and 5 mm., respectively) show sharply outlined thyroid plates. Accelerated growth in the area has thickened the plate and has entirely reduced the marked thyroid pit. There is obviously considerable crowding of cells in the active thyroid area, for cells are not only pushed down into the underlying mesenchyme but also there is invariably present at this stage a

very peculiar casting off of thyroid cells from the pharyngeal surface of the plate (figs. 2 and 3). This condition has been commented upon in a previous communication (Ramsay, '35). Coincident with the activity in the thyroid plate the mesenchyme underlying the floor of the pharynx increases noticeably in amount and migrates between the plate and the aortic sac (compare figs. 1, 2, 3 and 7). The thickened plate is not only countersunk by the continued growth of the mesenchyme (compare figs. 3 to 9) but also its area of attachment to the pharyngeal epithelium is markedly constricted (reduced), thus transforming the typical plate into a true diverticulum. Although great variation in contour normally occurs, newly formed diverticula are usually simple saccular in shape.

3. *Cavity formation.* a. Primitive thyroid follicle formation. After the usual shedding of cast off cells (figs. 2 and 3) the pharyngeal surface of the diverticulum is sharply defined (figs. 4 to 6). Soon, however, due to the growth activity of the thyroid epithelium, the walls of the diverticulum become folded out (evaginated) and in this way secondary diverticula (primitive thyroid follicles) as well as solid buds and sprouts of cells are produced peripherally from the original saccular anlage (figs. 3 to 6). The same crowding and sloughing of cells (already seen in the transformation of the plate into a diverticulum) accompanies the formation of secondary diverticula (compare figs. 2 and 3 with 4, 5 and 7). The cavities of the hollow buds thus formed quickly lose their connection with the main cavity. Indeed, the secondary outpouchings appear so quickly that without abundant material one is apt either to misinterpret the origin of their cavities or miss their time of formation entirely. Study of twenty-one embryos between 4 and 6 mm. in length shows that the cavities of the secondary diverticula (primitive thyroid follicles) were at first continuous with that of the main diverticulum from which they arose. These cavities, primitive thyroid follicles, are the ones to which our major interest is directed.

b. Intraglandular cavities (Norris). After the saccular anlage has given rise to the hollow buds and solid sprouts it

loses much of its residual lumen (usually by 8 mm.) producing a quite massive portion of thyroid cells in the midline (figs. 8 and 9). In establishing a more extensive contact with the surrounding vascular mesenchymal bed the thyroid cells separate in embryos 6 to 8 mm. in length to form tiny intercellular clefts (intraglandular cavities of Norris). The fate of the intraglandular cavities is always the same, to open at the outside of the anlage and to be invaded by mesenchyme which ultimately reduces the central massed region of the thyroid to plates and bands two cells in thickness. Figure 10 (15-mm. embryo) illustrates the result of two types of growth, 1) hollow and solid buds and sprouts laterally and dorsally (from early buds and primitive follicles) and 2) plates of cells in the midline (produced by intraglandular cavities).

4. *Prefollicular stage.* Although this period of thyroid development embraces a considerable period of time, it can be passed over with but a few statements. Complex IV is brought into contact and fuses with the thyroid anlage when the embryos are 12 to 13 mm. long. Since this discussion is concerned primarily with thyroid parenchyma, no mention will be made of the ultimobranchial body or its fate except to state here that if not by position alone one can define the boundary of the ultimobranchial body reasonably accurately by its lack of mitotic figures, a characteristic feature beginning with the time of fusion.

Primitive follicles (hollow buds) are retained as development proceeds and occur most numerous in the lateral and dorsal portions of the anlage (lateral lobes) while the central region (isthmus) contains but few and is composed almost entirely of plates and bands which, beginning to branch, are becoming irregular (fig. 10). Figures 11 and 12 illustrate the appearance of primitive follicles in the lateral lobes of fetuses 28 and 35 mm. in length, respectively. It will be noticed that while at first the walls of the primitive follicles are but one cell in thickness (figs. 5, 6, 8 and 10) they become many cells in thickness later in development (figs. 11 and 12). It is clear however that only the single layer of cells bounding

the lumen shows any definite arrangement and also that the outlying cells have originated from the former by mitotic proliferation.

The gland as a whole immediately prior to the appearance of colloid (40 mm.) may be briefly described as being composed of irregularly branching bands and buds (strands) of thyroid parenchyma, highly vascularized and quite compact (figs. 11, 12 and 13). Many primitive follicles are present.

5. *Follicular stage.* The hitherto empty primitive follicles, indeed the only follicles so far existent, contain small amounts of a highly refractile, acid-staining substance by the time fetuses reach 40 to 41 mm. in length. Figure 13 illustrates the appearance of the primitive follicles at the initiation of colloid formation (or storage) in a 41-mm. fetus. By 50 mm. the colloid has increased in amount and the formerly flattened, slit-like primitive follicles present a somewhat rounded contour (figs. 14 and 15). With the appearance of colloid, it is proper to term the cavities designated above 'primitive follicles' as 'primary thyroid follicles.' 'Primitive' is employed merely to emphasize their precocity in development and to distinguish them arbitrarily from the 'primary' follicles to be described subsequently. Shortly after colloid appears in primitive follicles it is demonstrable (50 mm.) as tiny droplets scattered apparently indiscriminately throughout the remainder of the dense irregularly packed thyroid material. The cells bounding such droplets have at first no regular orientation, such as is usual for follicular cells (figs. 14, 15 and 16), but later with the increase in size of the colloid globules the related adjacent cells become arranged typically and the characteristic picture of adult thyroid parenchyma is established. These follicles are formed completely apart from the primitive follicles described previously, and, since they correspond to the 'primary' follicles of most other animals, they are so termed here. While primary follicles of the human thyroid gland possess lumina before colloid is produced in them (Kingsbury, '15; Norris, '16), the reverse is true in the cat.

Primitive follicles can be easily traced into their later development as well as into the adult stage by the following identifying features: 1) their size, 2) their peculiar shape, and 3) by the staining character of their nuclei. Figure 20 illustrates relatively enormous primitive follicles (115-mm. fetus) with quite regular contours. On the other hand the primitive follicles shown in figures 17 and 18 (150 mm.) are peculiar for their great length, for when followed in serial sections these elongated follicles resemble long cylinders with very few irregularities along their entire extent. Those in figure 19 (150 mm.) are of interest because of their great irregularity in outline. Since the nuclei of primitive follicle cells stain darkly and are quite dense, it is possible to identify them with certainty even when in the midst of ordinary primary and secondary follicles (compare figs. 14 to 21).

Secondary follicles were observed to be formed by 1) constriction or division of primitive and primary follicles (figs. 14 and 17 to 19), and 2) by direct hollow outgrowths from existing follicles followed by constriction. It is questionable whether follicles formed from solid epithelial outgrowth or appendages (later gaining a lumen) of primary and primitive follicles are correctly interpreted as being secondary follicles. In the majority of cases more justification is seen for terming them 'primary' rather than 'secondary,' if such a distinction must be made. Not all of the thyroid parenchyma is employed immediately in the formation of follicles for some retains a marked tendency for growth and continues to add thyroid material for some time following birth.

DISCUSSION AND SUMMARY

The first visible indication of thyroid development is the sudden acceleration of cell division which is evidenced by the appearance of numerous mitotic figures and a thickening of the epithelium in the primitive thyroid area (plate). It is difficult to detect or interpret the factors which initiate this abrupt activity. There is reason to believe, however, that the determination of thyroid occurs some time before the

morphological relations of the primitive pharynx have been established. Willier, Rawles and Rudnick ('31), using the chorio-allantoic grafting method, found that the anterior two-thirds of the pellucid area of primitive-streak stage chick blastoderms when split in two by a median cut and grafted, produced heart, liver, and thyroid for both right and left sides. Their "results demonstrate the presence of two laterally-situated areas of prospective thyroid, liver, and heart in the blastoderm anterior to the knot (head process) and along the sides of the knot (definitive primitive-streak stage)." The grafts which produced thyroid invariably produced heart also. Rudnick ('32) employed the same technique in studying further the anterior portion of the chick's blastoderm. Among her conclusions the following (p. 312) appears.

Right, median, and left pieces of the anterior end of the pellucid area of definitive primitive-streak and head-process blastoderms were studied with reference to their capacity for producing thyroid when grafted. Grafts of the thyroid of the 72-hour chick were also observed. Thyroid is found in all three types of grafts from the primitive-streak and early head-process stages; after the formation of the head fold, in lateral pieces only. This may indicate a) the gradual lateral separation of two distinct centers, originally near the median line; or b) the progressive bilateral localization of what was in the beginning a single broad field.

The above studies suggest to the present writer the possible role of the primitive blastodermic mesoderm in thyroid induction.

The shape of the early thyroid diverticulum is obviously due to three growth conditions present: 1) first and of most importance, the rapid growth of the thyroid material itself, 2) the morphology of the propharyngeal region, and 3) the growth activity and consequently the mechanical effect of mesenchymal material surrounding the thyroid plate. The rapid growth of the thyroid plate has already been referred to in the foregoing paragraph and requires no further comment here. The morphology of the pharynx is likewise an important factor. There is evident at first in the pharyngeal

floor a mid-ventral groove which, acted upon by the growth of the plate, tends to favor diverticulum formation. A fairly flat pharyngeal floor would not facilitate the production of a diverticulum, but on the other hand, a single solid mass (bud) or solid strands and masses of material are given off from the plate into the underlying tissue. The role of mesenchymal material in shaping the anlage is related most intimately to the two above-mentioned conditions. Since at first the plate rests directly on the aortic sac it is impossible for diverticulum formation to occur unless the periphery of the plate is elevated and constricted; herein lies the major mechanical role of the mesodermal material (compare figs. 1 to 9). Utilizing many embryos of similar developmental stages one finds individual structural variations, particularly in the early morphology of the primitive pharynx. Necessarily, therefore, there are many variations in the appearance of the early thyroids buds and diverticula. Norris ('18 b) has very carefully tabulated and illustrated these variations for the human (p. 448). Thyroid diverticula in the cat differ from those of the human only in that the former invariably possess lumina in the thyroid portion proper.

The origin of 'primitive follicles' directly from the open thyroid diverticula appears to be a process peculiar to the cat. Remak (1855) set forward the theory that a primary saccular anlage in the chick gives rise to secondary follicles by constriction or budding. Bradway ('29), however, has not given the same interpretation for the chick. Frazer and Hill ('15), studying the development of the pharyngeal derivatives in the Marsupialia reported a hollow thyroid diverticulum, the cavity of which disappeared and later in its place a number of slits appeared. These authors were able to trace the 'slits' up to 52-mm. pouch young. Some slits contained an 'acid-staining material' referred to as a 'secretion' but obviously not to be interpreted as colloid for they believed colloid formation not yet to have been initiated at that time (52 mm.). Shaner ('21) described 'slits' which appear in thyroid anlagen of 9.5-mm. turtle embryos, having arisen within a solid diverticulum (not saccular as in the cat). Shaner noted that

the slits in a 16-mm. turtle embryo contained an 'acid-staining secretion.' I consider the 'slits' of the Marsupialia and turtle described above to be similar to the 'primitive follicles' of the cat but differing in possessing apparently no demonstrable relation to the cavity (if present) of the early thyroid diverticulum.

For the interpretation of the fundamental nature of the thyroid gland it is very significant that the first buds or sprouts (secondary diverticula, primitive thyroid follicles) originating from the cat's thyroid diverticulum carry with them a portion of the cavity of the parent diverticulum (figs. 4, 5 and 6). With such a fortunate condition prevailing, it is obvious that the inner surface of the primitive follicles represents and is a part of the original pharyngeal surface of the thyroid plate. Expressed differently, the inner surface of the primitive follicles is essentially a free surface and, considered phylogenetically, it is a free glandular (endostyle) surface. As was expected, therefore, colloid was present in the primitive follicles before it was demonstrable elsewhere (figs. 13 and 14) and, indeed, before any other follicular cavities are formed.

As is well known, the thyroid gland makes its phylogenetic appearance during the metamorphosis of the larval or Ammocoetes stage to the adult lamprey. The coincident degeneration of the endostyle organ results in the reduction of the long coiled tubes to the smaller closed vesicles (thyroid follicles) which have lost their connection (duct) with the floor of the pharynx (Marine, '13 a and b). Therefore, in the first place the thyroid was developed from cells which constituted a part of an exocrine gland. Whether these cells originally took part in the exocrine activity of the endostyle or not is beside the point here emphasized. It is at least doubtful that the thyroid gland of the lamprey is as adaptive or as specific as that of higher forms for two reasons, first, because the thyroid hormone(s) has not yet been demonstrated therein, and secondly, many of the cells comprising the thyroid follicles of the lamprey retain their true cilia which they possessed while a part of the endostyle (Marine,

'13 a). Cowdry ('21), describing a single flagellum on each cell in the thyroid vesicles of the dogfish, writes (p. 295), "We may at least conclude that the presence of flagella extending from the cell into the follicular cavity indicates that this cavity originally communicated with the pharynx; in other words that the flagella constitute definite clues to the primary polarity of the cells." Gudernatsch ('11) has given an excellent interpretation of the thyroid of higher forms on the basis of "changes in function producing changes in the character and uniformity of organs." Cognizant of the difficulties encountered in making statements which attempt to bridge such a gap as that existing between the thyroids of mammals and of the lamprey, the evidence provided by the present study permits at least one fundamental comparison to be drawn, to wit: Since it is possible to trace the 'primitive follicles' of the cat directly from the hollow diverticulum and because they possess a colloid first, they may be compared to the original (primary) follicles left by the degenerating endostyle organ (gland). The glaring discrepancy in thyroid development in the various forms is, in general, the absence of structural manifestations which permit such parallelisms to be observed. It must be admitted, also, that here only the primitive follicles (which later become primary follicles) of the cat exhibit this close relationship to the parent saccular diverticulum. The difficulty in interpreting the origin of thyroid follicles is apparently due to a necessarily greatly prolonged and intensified growth period which results either in slurring over and thereby obscuring the more fundamental original growth relationships or in instituting entirely new growth patterns to compensate for 1) the change in the intensity and duration of the long growth period, and 2) the change in the character of the gland. While in most forms the latter is undoubtedly the case, the growth pattern of the cat's thyroid demonstrates the presence of the former also.

Many different opinions concerning the character of the growth pattern of thyroid parenchyma have been advanced. The early investigators tended to misinterpret the actual

structure of the growing gland for it was variously described as being composed of 1) cords and cylindrical tubes, 2) cell masses and cords, 3) tubules, 4) tubules and acini or tubulo-acinar, and 5) branched tubules 'as in a salivary gland.' Norris has shown most of these to be incorrect. From the results of the present investigation it becomes obvious that the thyroid in its growth may utilize more than one means of cell arrangement and that, in a general way, the entire thyroid grows according to the growth pattern(s) characteristic of its species. These are: 1) growth by way of plates and bands generally one cell thick (calf), 2) plates and bands irregularly branched and two cells thick, and 3) growth by buds and sprouts from plates and/or bands or from the diverticulum directly. A fourth method may be mentioned, a combination of any of the above, a good example of which is found in the cat. As seen from the foregoing description, growth at first is accomplished by buds and solid sprouts alone (figs. 5, 6 and 7) while later, by the appearance of intraglandular cavities, the central portion of the anlage is reduced to plates and bands two cells in thickness, the buds and sprouts being restricted to the lateral and dorsal regions only (fig. 10). This growth pattern holds through the rest of morphogenesis and into the follicular epoch. Although a great mass of thyroid gland is provided by the early intense growth period, thyroid material is still added until after birth. This is accomplished by formation of secondary follicles (from primary follicles) and by growth and differentiation of material itself not at the time constituting a follicle (figs. 17, 18 and 19).

There is some controversy over the presence of thyroid lobules. Although I have found nothing as elaborate as the lobulation and gland units described by Williamson and Pearse ('23) there is definite indication of lobulation within the thyroid gland. As was described in detail elsewhere in this paper, the early thyroid mass becomes dispersed into many subdivisions by whatever growth pattern(s) are employed. From these more or less primary divisions of the mass all of the thyroid material must develop. With the

subsequent expansion of the material by growth the primary divisions are apparently more or less obliterated but none the less still present (figs. 13 to 16) as can be evidenced, if carefully examined, by the presence of delicate connective tissue septa. In the thyroids of most adult forms sufficient amounts of connective tissue are present to outline at least some degree of lobulation.

In the cat a very fortunate peculiarity makes the lobulation very clear. The mature cat's thyroid possesses a lymph sac which is surprisingly complete, surrounding the entire thyroid except at the points of entrance and exit of blood vessels, etc. (figs. 17 to 21). The development of this lymph sac can be followed if figures 12, 13 and 17 to 21 are examined and compared. One is immediately reminded of the condition in some fishes (Ferguson, '11, and others). It will be seen from study of figures 17 to 19 that the lymphatic endothelium penetrates the lobules to a marked degree, indeed it appears that each follicle has broad contact with lymphatic endothelium. Such a condition is explicable only by an appreciation of the close relationship existing between reticular tissue (the important stromal tissue of the thyroid) and endothelium, reticulo-endothelial cells, littoral cells, etc., in general. The endothelium of the peri-thyroid lymph sac and its intra-lobular ramifications bears the same intimate relation to the follicle cells as do the blood capillaries elsewhere. The possible significance of the lymph sac in transporting thyroid secretion immediately arises.

Hicks ('26), studying the secretory path of the thyroid gland of dogs, concludes that "Thyreoglobulin, as an index of thyroid secretion, passes from the thyroid gland to both lymphatics and veins, chiefly by the latter route." The present study strongly indicates that there exists within the thyroid gland of the cat a more intimate and extensive relation between the follicle cells and the lymphatics than is present in other mammals (figs. 17 to 21). Consequently one would expect the lymphatics to be of greater importance in receiving and transporting thyroid secretion in the cat.

Experimental confirmation of this developmental study has not, in the writer's knowledge, been attempted.

Williamson and Pearse ('23) believe the human thyroid gland to be divided into lobules and the lobules into 'gland units,' the latter completely circumscribed by connective tissue. Within each such gland unit they describe a 'lymph space' which is peculiar in that it possesses but one wall (outer) while the contained lymph bathes the follicle cells of the unit directly. In 1930 the same authors again present their original speculations and add a discussion of a 'closed thyreo-thymus lymph system.' I have been unable to substantiate their descriptions after a personal study of the human thyroid as well as that of the common domestic animals. Instead, it appears certain that the 'lymph space' figured on page 532 (Williamson and Pearse, '30) is a typical cystic tract or 'duct' lined with desquamating epithelium of pharyngeal entodermal origin instead of lymphatic endothelium as Williamson and Pearse insist. I feel sure that the many current studies of thyroid, parathyroid, and thymus development explains such 'spaces' on a basis entirely different than that employed by Williamson and Pearse.

In regard to the peculiarities of the lymph drainage of the thyroid the following investigations may be mentioned: Caylor, Schlotthauer and Pemberton ('27) and Mahorner, Caylor, Schlotthauer and Pemberton ('27) have shown by injection prior to embalming, followed by careful dissection, that the lymph drainage from human and dog thyroids is to cervical lymph nodes and thence to cervical lymph trunks and, also, that some lymph vessels proceed directly into veins without first traversing lymph nodes. Chouke, Whitehead and Parker ('32) also disprove the statements of Williamson and Pearse and agree with the findings of Caylor and his associates. They do not, however, report any lymph drainage from the thyroid directly into veins (without intervening nodes) but mention small veins from the thyroid which 'resemble lymph vessels.' Polonskaja ('34) reports at least one case (human) wherein thyroid lymphatics empty directly into the

jugular vein. When studying the thyroid lymph drainage in the cat by use of serial sections it is clear that, although the chief drainage is to cervical nodes, some small vessels enter the great veins directly.

CONCLUSIONS

1. The hollow 'primitive follicles' of the cat's thyroid spring directly from the saccular diverticulum by budding and constriction.

2. The mode of primitive follicle formation in the thyroid of the cat furnishes non-speculative developmental evidence that the primary polarity of the follicle cells is toward the follicular cavity.

3. The obscure methods of follicle formation usually found in thyroid morphogenesis are interpreted to be due to a slurring over of the fundamental growth relationships and the introduction and utilization of entirely new growth patterns to meet and compensate for changes in the intensity of the long growth period.

4. The different growth patterns of thyroid parenchyma are discussed.

5. A peri-thyroid lymph sac together with its possible role in transporting thyroid secretion is described in the cat.

LITERATURE CITED

- BORN, G. 1893 Ueber die Derivate der embryonalen Schlundbogen und Schlundspalten bei Säugetieren. *Arch. f. mikr. Anat.*, Bd. 22, S. 271-318.
- BRADWAY, WINNEFRED 1929 The morphogenesis of the thyroid follicles of the chick. *Anat. Rec.*, vol. 42, pp. 157-167.
- CAYLOR, H. D., C. F. SCHLOTTHAUER AND J. DE J. PEMBERTON 1927 Observations on the lymphatic connections of the thyroid gland. *Anat. Rec.*, vol. 36, pp. 325-333.
- CHOUKE, K. S., R. W. WHITEHEAD AND A. E. PARKER 1932 Is there a closed lymphatic system connecting the thyroid and thymus glands? *Surgery, Gynecology and Obstetrics*, vol. 54, pp. 865-871.
- COWDRY, E. V. 1921 Flagellated thyroid cells in the dogfish (*Mustelus canis*). *Anat. Rec.*, vol. 22, pp. 289-299.
- FRASER, E. A. 1915 The development of the thymus, epithelial bodies, and thyroid in the Marsupialia. Part II. *Phascolarectos*, *Phascolomys*, and *Perameles*. *Phil. Trans. Roy. Soc., London*, series B, vol. 207, pp. 87-112.

- FRASER, E. A., AND J. P. HILL 1915 I. The development of the thymus, epithelial bodies, and thyroid in the Marsupialia. Part 1. *Trichosurus vulpecula*. Phil. Trans. Roy. Soc. London, Series B, vol. 207, pp. 1-85.
- FERGUSON, J. S. 1911 The anatomy of the thyroid gland of Elasmobranchs, with remarks upon the hypobranchial circulation in these fishes. *Am. J. Anat.*, vol. 11, pp. 151-210.
- GODWIN, M. C. 1936 The early development of the thyroid gland in the dog with especial reference to the origin and position of accessory thyroid tissue within the thoracic cavity. *Anat. Rec.*, vol. 66, pp. 233-251.
- GUDERNATSCH, J. F. 1911 The thyroid gland of the teleosts. *J. Morph.*, vol. 21, pp. 709-782.
- HICKS, C. S. 1926 Innervation and secretory path of the thyroid gland. *J. Physiol.*, vol. 62, pp. 193-202.
- KINGSBURY, B. F. 1915 The development of the human pharynx. I. The pharyngeal derivatives. *Am. J. Anat.*, vol. 18, pp. 329-397.
- MAHORNER, H. R., H. D. CAYLOR, C. F. SCHLOTTHAUER AND J. DE J. PEMBERTON 1927 Observations on the lymphatic connections of the thyroid gland in man. *Anat. Rec.*, vol. 36, pp. 341-348.
- MARINE, DAVID 1913 a The evolution of the thyroid gland. *Bull. Johns Hopkins Hosp.*, vol. 24, pp. 135-141.
- 1913 b The metamorphosis of the endostyle (thyroid gland) of *Ammocoetes branchialis* (larval landlocked *Petromyzon marinus* [Jordan] or *Petromyzon dorsatus* [Wilder]). *J. Exp. Med.*, vol. 17, pp. 379-395.
- NORRIS, E. H. 1916 The morphogenesis of the follicles of the human thyroid gland. *Am. J. Anat.*, vol. 20, pp. 411-448.
- 1917 The early morphogenesis of the thyroid gland in *Squalus acanthias*. *Anat. Rec.*, vol. 11, pp. 391-392.
- 1918 a The morphogenesis of the thyroid gland in *Squalus acanthias*. *J. Morph.*, vol. 31, pp. 187-223.
- 1918 b The early morphogenesis of the human thyroid gland. *Am. J. Anat.*, vol. 24, pp. 443-465.
- POLONSKAJA, R. 1934 Die Lymphgefäße der Schilddrüse und ihr Zusammenhang mit dem Venensystem. *Folia Anat. Japonica*, Bd. 12, S. 311-317.
- RAMSAY, A. J. 1935 The development of the palatine tonsil (cat). *Am. J. Anat.*, vol. 57, pp. 171-203.
- REMAK, R. 1855 Untersuchungen über die Entwicklung der Wirbelthiere. G. Reimer, Berlin. S. 122-123.
- RUDNICK, DOROTHEA 1932 Thyroid-forming potencies of the early chick blastoderm. *J. Exp. Zool.*, vol. 62, pp. 287-317.

- SHANER, R. F. 1921 The development of the pharynx and aortic arches of the turtle, with a note on the fifth and pulmonary arches of mammals. *Am. J. Anat.*, vol. 29, pp. 407-429.
- WILLIAMSON, G. S., AND I. H. PEARSE 1923 The structure of the thyroid organ in man. *J. Path. and Bact.*, vol. 26, pp. 459-469.
- 1930 The anatomy (comparative and embryology) of the special thyroid lymph system, showing its relation to the thymus; with some physiological and clinical considerations that follow therefrom. *British J. Surg.*, vol. 17, pp. 529-550.
- WILLIER, B. H., M. E. RAWLES AND D. RUDNICK 1931 Bilateral localization of prospective heart, liver and thyroid in the early chick blastoderm as studied in chorio-allantoic grafts. *Anat. Rec.*, vol. 48 (Suppl.), p. 68.

PLATE 1

EXPLANATION OF FIGURES

1 Sagittal section through the pharyngeal floor of a 25-somite cat embryo. No mesenchyme intervenes between the slightly depressed thyroid plate and the endothelium of the aortic sac. Many mitotic figures are seen in the plate area. The oral extremity is at the left. $\times 150$.

2 and 3 Thyroid plates from embryos 4 and 5 mm. in length, respectively. Note the shedded material on the pharyngeal surfaces of the thyroid plates. Mesenchyme now underlies the plate areas. $\times 150$.

4 Transverse section through the thyroid diverticulum of a 7-mm. embryo showing an unusual variation in the shape of the early diverticulum and also the origin of two primitive follicles. $\times 150$.

5 Sagittal section showing an advanced diverticulum (5 mm.) from which secondary diverticula (primitive follicles) are being formed. The smaller diverticulum has just closed off from the parent diverticulum leaving a slight cleft to mark its former relation. Followed laterally, two more diverticula broadly connected to the parent cavity are found. $\times 150$.

6 Sagittal section of another 5-mm. cat embryo more advanced than that figured above. This illustration demonstrates again the open diverticulum and primitive follicle formation, also the origin of solid buds and sprouts. $\times 150$.

7 Thyroid of a 5.5-mm. cat embryo seen in sagittal section. The diverticulum has just closed but the original lumen is always retained. The primitive follicles all lie lateral to this midline section. Solid buds are being formed and an 'intraglandular cavity' has appeared (indicated by the arrow). $\times 150$.

8 This 5-mm. embryo is developmentally older than the preceding. Note the caudal displacement of the thyroid and its intimate relation to the aortic sac. A primitive follicle (probably the residual lumen of the diverticulum) is seen in the lower center. An 'intraglandular cavity' (Norris), indicated by the arrow, is already opening to the outside of the gland mass to be invaded by mesenchyme. $\times 150$.

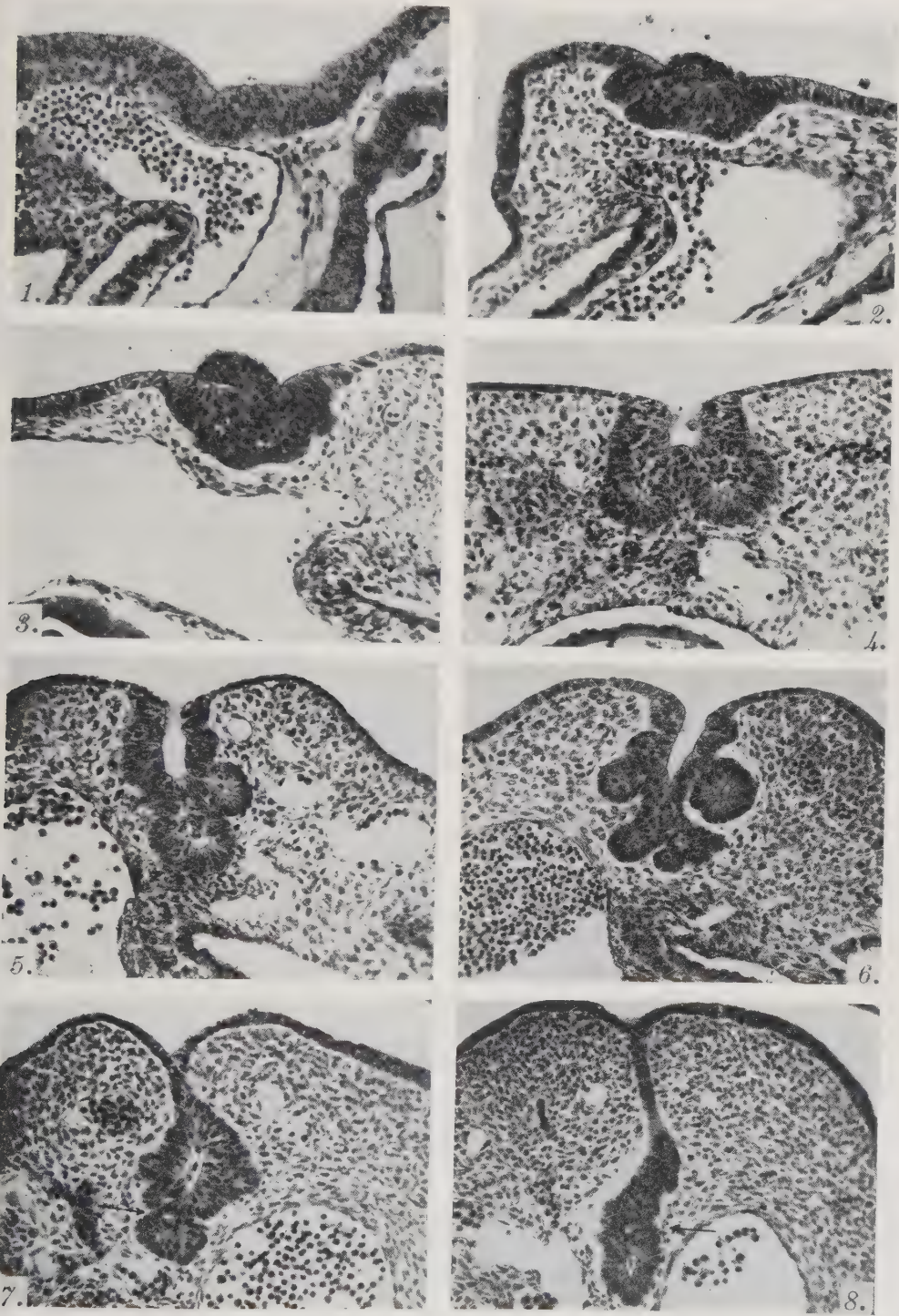


PLATE 2

EXPLANATION OF FIGURES

9 Mid-sagittal section of a 6-mm. cat embryo to illustrate an intraglandular cavity. The thyroid stalk has just broken. Note that the nuclei lie next to the cavity, the reverse of the condition obtaining in thyroid follicles. $\times 150$.

10 Transection of a 15-mm. embryo. Note the centrally located (isthmus) plates two cells thick while buds and solid sprouts and many primitive follicles are laterally located. Complex IV has fused with the lateral lobes on either side. $\times 50$.

11 Lateral lobe of a 28-mm. cat embryo which illustrates 1) primitive follicles (arrows), and 2) proliferation of cells from their walls. Increased vascularity is characteristic by this stage. $\times 150$.

12 Lateral lobe in a 35-mm. cat embryo as seen in frontal (horizontal) section, the trachea being at the left. Parathyroid III is seen above; parathyroid IV, thymus IV (light area), and related strands of ultimobranchial body material are seen centrally located. Arrows indicate primitive follicles. Slight tissue clefts (L.C.) foretell the formation of the lymph sac. $\times 50$.

13 This peripheral thyroid material from a 41-mm. cat fetus shows primitive follicles containing small amounts of an acid-staining highly refractile secretion which is considered here to be colloid. The 'primary' follicles of other animals are not present here as yet. Note the clear central area of the follicle cells. The lymph cleft seen at the left is rapidly surrounding the lobules. Its endothelial lining is clearly seen. $\times 270$.

14 Primitive (primary) follicles now contain much colloid (50-mm. cat). Note the darkly-staining nuclei of the follicle cells and also the apparent constriction of the follicle. Primary follicles (comparable to those of other animals) are indicated by the arrows. Note the light staining nuclei and their irregular placement in the latter follicles. $\times 270$.

15 and 16 Primary follicles of cat fetuses of 50 and 53 mm. respectively. Notice the irregular follicle cell arrangement as compared to that of the primitive follicles. $\times 270$.

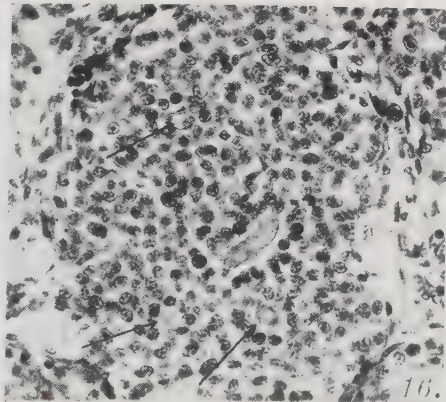
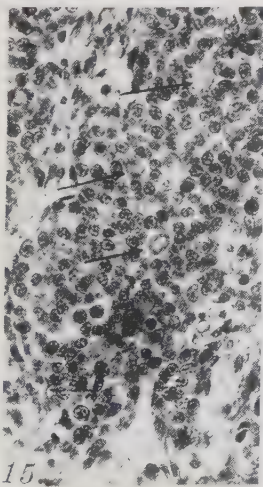
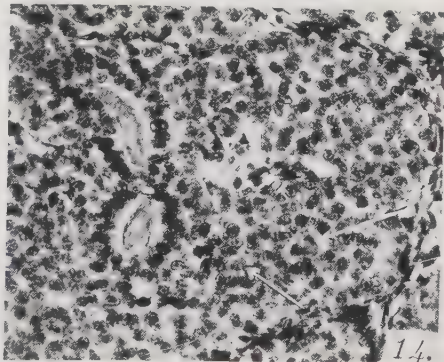
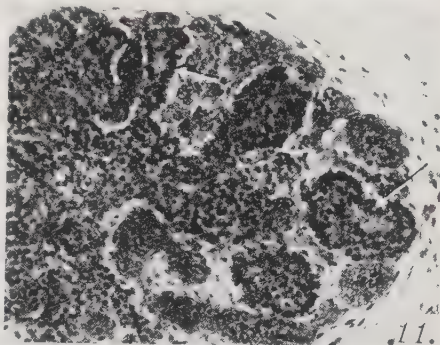
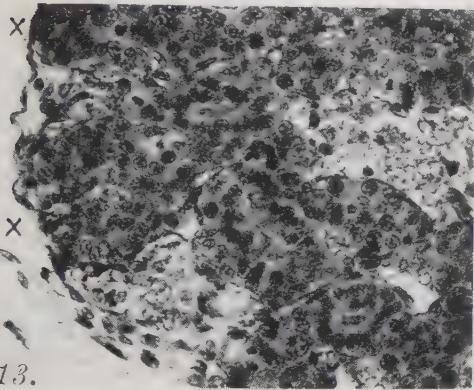
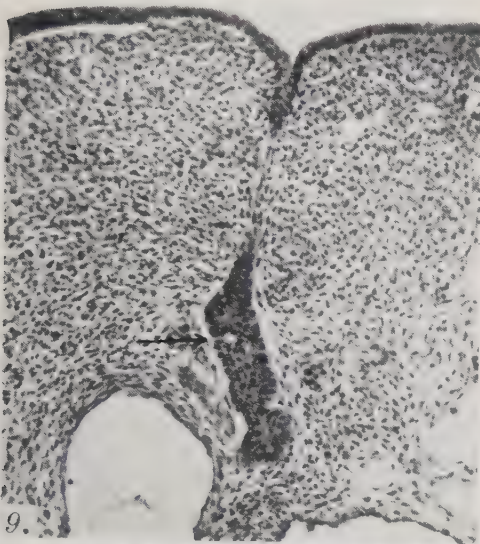


PLATE 3

EXPLANATION OF FIGURES

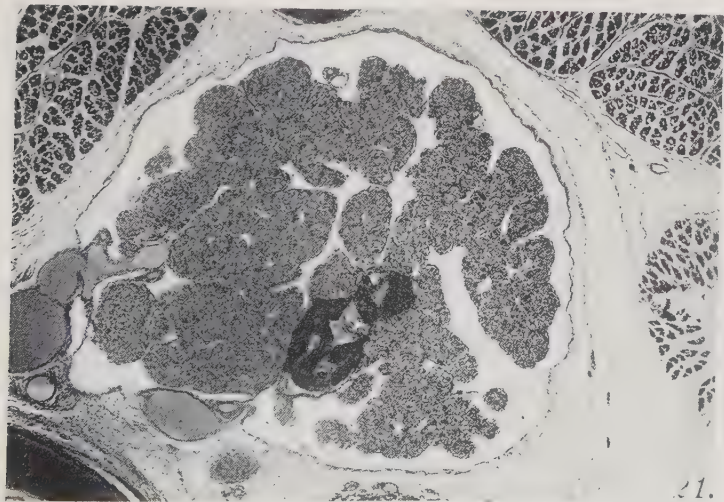
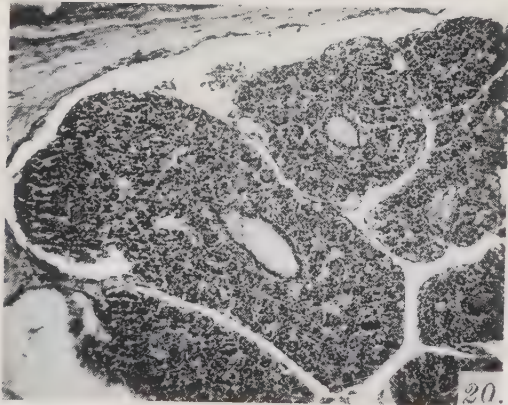
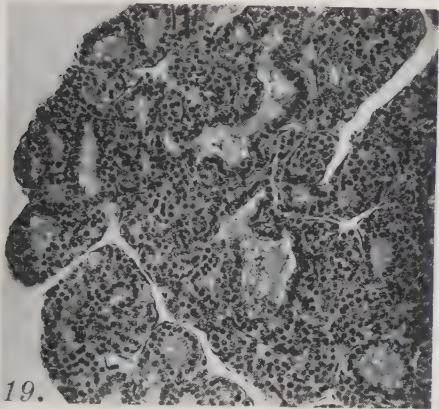
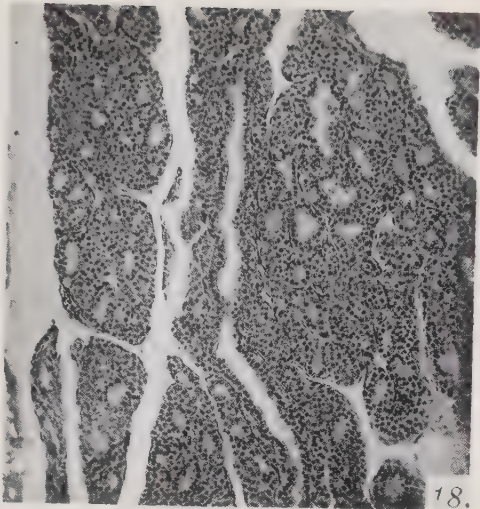
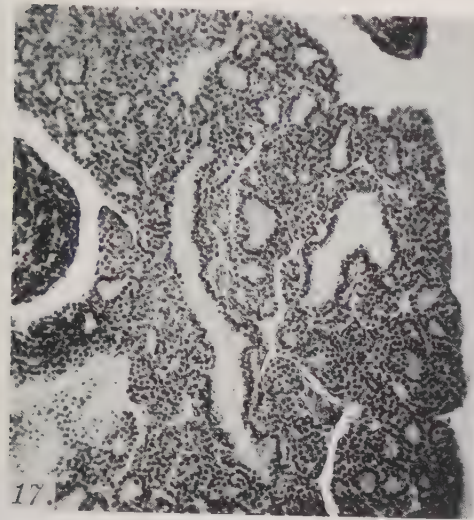
17 Large primitive follicles in a 150-mm. kitten's thyroid. A prominent lymph cleft is also apparent. Numerous solid masses of cells present no follicular formation as yet. $\times 150$.

18 A long primitive follicle in the thyroid of a 150-mm. kitten. When followed serially this follicle is found to possess over twice the length it shows in this illustration. Note the lymph cleft. $\times 150$.

19 Irregular primitive follicles in 150-mm. kitten which are evidently giving rise to secondary follicles by budding and constriction. Notice that the peri- and intralobular lymphatic endothelium lies directly upon the follicular epithelium (lower center), as seen also in figures 17 and 18. $\times 150$.

20 Thyroid of a 115-mm. cat fetus to illustrate large primitive follicles and the thyroid lobulation, the latter made prominent by the lymph cleft. $\times 75$.

21 Lateral thyroid lobe of 120-mm. cat fetus. This photograph is included to illustrate the very characteristic lymph sac which makes the thyroid lobulation very apparent. Parathyroid IV and thymus IV are in the lower center; a vein and the dorsolateral border of the trachea are seen at the lower right. $\times 30$.



IMPROVED HISTOCHEMICAL METHODS FOR CHLORIDE, PHOSPHATE-CARBONATE AND POTASSIUM APPLIED TO SKELETAL MUSCLE

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Current histochemical methods for chloride, phosphate, carbonate and potassium can be traced back through Macallum to much earlier work ('08). That these methods suffered from fundamental errors is recognized tacitly by many histologists and is borne out by the paucity of sound scientific contributions derived with their use and by the failure of present day workers to apply them to the elucidation of biological problems. The essential difficulty in the techniques is that they all permit diffusion of water-soluble substances. In addition, the procedures for these histochemical tests failed to take advantage properly of the great sensitivity of reagents for these ions, and the results were not as delicate as they could be. The subtle sources of error are enumerated in great detail by Lison in his handbook and in a more recent paper on the localization of certain ions in gastric glands. The methods introduced in this paper for the identification of chloride, phosphate-carbonate, and potassium are designed to overcome or minimize these difficulties with the aid of recent technical advances.¹ Numerous controls were made at each step to assist in preventing or reducing diffusion without sacrificing unduly the sensitivity of the reactions. The methods

¹ The term 'phosphate-carbonate' is used advisedly to indicate that the method in use demonstrates both of these anions when they are present in large enough amounts, without distinguishing between them; the expression does not refer to the complex salts of phosphate and carbonate such as are present in bone.

enumerated above have been applied systematically to an investigation of their distribution in certain glands and in skeletal muscle. In this paper only observations on the latter are recorded and discussed.

Recently, in connection with the broader problems of membrane permeability and maintenance of osmotic equilibrium in cells, attempts have been made by physiologists and biochemists to estimate quantitatively the inorganic constituents present within the skeletal muscle cell. All of these estimates are based on chemical analyses of the entire muscle, that is, of a complex tissue which includes muscle cells, connective tissue, extracellular tissue fluid and such additional structures as blood vessels and their contents. Two assumptions have been made in all the more recent studies: 1) the composition of the tissue fluid bathing the muscle cell is almost identical with that of the blood plasma; 2) no chloride is present within the muscle fiber—the total amount remaining extracellularly in the tissue fluid. Applying these two assumptions to chemical analyses of whole muscles, calculations have been made of the concentrations of certain inorganic substances within the muscle cell. The most extensive results have been obtained by Fenn working particularly on the sartorius muscle of the frog. He finds that potassium, phosphate and bicarbonate are present within the muscle fiber, in contrast with the assumed complete intracellular absence of chloride. His calculations show that, volume for volume, the intracellular:extracellular ratio is about 30:1 for potassium, a minimum of about 3:1 for inorganic orthophosphate, and about 1:4 for bicarbonate.

Such marked differences between intra- and extracellular phases should be easily demonstrable with adequate histochemical methods. The results recorded in this paper are, within the limits of the method, in remarkable agreement with the conclusions noted above. Chloride cannot be detected within the muscle fiber even under conditions when the chloride content of the whole muscle is greatly increased. The anion is, however, easily visible in the connective tissue surrounding the muscle fibers. Phosphate and carbonate together are

present in appreciable amounts in the muscle cell. Potassium is present in the muscle fiber in amounts vastly greater than that which can be seen in the interstitial connective tissue. These observations were made on the frog sartorius muscle treated experimentally in much the same way as Fenn and his co-workers describe in their series of papers, with the object in view of utilizing their comparable quantitative data in the interpretation of the results.

METHODS

The essential features of the methods are: 1) The retention of substances in or very close to their original inter- and intracellular sites by the use of the Altmann freezing-drying technique. 2) The sectioning of the frozen-dried material (embedded in paraffin) at a definite thickness in order to make possible rough comparative studies, to control more rigidly the conditions of the subsequent procedure, and to permit ready access of the reagents to the cells. 3) The removal of the paraffin by petroleum ether to allow the silver reagents to penetrate the section. 4) The use of silver nitrate solutions designed to precipitate specifically chloride ions, alone or together with phosphate and carbonate ions, and of a solution of sodium cobalti-nitrite to visualize potassium specifically. 5) The reduction of the precipitated silver salts by exposure to an arc light in order to make them visible.

The details of the method are as follows: Tissues or pieces of organs are frozen in liquid air and dried in a vacuum at temperatures varying from -60 or -62°C. to -79°C. After complete dehydration they are transferred to Grüber's paraffin (m. p. 50° to 52°C.), infiltrated in vacuo for 10 to 15 minutes at temperatures up to 60°C. , and embedded. During the transfer from the vacuum tube to the paraffin, strict precautions are taken to prevent condensation of water on the dried tissues. Just before use, the paraffin is heated in a vacuum at 100°C. or higher for 15 minutes or until bubbling stops. Sections 15μ thick are cut and these are mounted toward one edge on chemically clean large cover slips. They

are pressed down by the finger and after just melting over a very small flame are pressed down again. It has been found repeatedly that no error is introduced in pressing the section down with the finger. At this point sections to be tested for potassium are removed to the cold room, where they are treated in a manner that will be described later (p. 315). The subsequent treatment of sections to be studied for chloride and phosphate-carbonate proceeds in the following way:

The sections attached to the cover slip are submerged in a watch glass filled with anhydrous petroleum ether (b. p. = 20° to 40°C.), freshly distilled over sodium. The petroleum ether is replaced frequently. The watch glass is covered with another at all times except during the actual manipulations. When the paraffin is dissolved, the cover slip with the section attached is removed rapidly and a flame is applied immediately. The petroleum ether is burnt off and the warm cover slip is allowed to cool. With a little practice, the cover slip can be manipulated in such a way that the section does not slip off or become charred during this process. When the section approaches room temperature it is covered by a drop of one of the silver reagents.

Reagent 1 consists of a 60% solution of silver nitrate diluted with a sufficient quantity of concentrated phosphoric acid to prevent the precipitation of rather large concentrations of phosphates and then saturated with silver chloride; after filtering, 2 to 3 drops of water are added to every 10 cc. of reagent. Reagent 2 consists of a 60% solution of silver nitrate saturated with silver phosphate and silver chloride; after filtering, it is diluted with water as above. The reagents are kept tightly stoppered and in the dark except when in use. They are filtered just before using.

The fluid is decanted from the cover slip after a few seconds. A drop of chemically pure glycerine is placed over the section on the cover slip, which is then reversed over a chemically clean slide. The sections thus prepared in doublets are simultaneously exposed to a carbon arc radiation for the same time interval. The distance between the carbon arc and the slide is such that the section is not warmed during the exposure.

The sections last for a short time and it is best to study them directly after the radiation is completed. They may be examined by transmitted light or with dark field illumination. The reduced silver representing chloride, or phosphate-carbonate, or both, as the case may be, is visible as a yellow to brown, diffuse, homogeneous coloring with or without brown or black particles. With the dark field, the diffuse colorations are in most cases analyzed into finely particulate, reduced silver granules appearing usually orange or rust colored; also by this method the granules visible as such by transmitted illumination are more easily differentiated from the background. The highest power lens used with the dark field condenser was the 4 mm. high-dry apochromat with a numerical aperture of 0.95. The light required for the use of an oil immersion lens is too intense to be used with safety on these preparations because of its great reducing capacity.

The first reagent precipitates specifically the maximal concentration of chloride that can be detected by this method in the presence of phosphate and carbonate ions. Phosphate is kept in solution due to the excess phosphoric acid added to the reagent; carbonate is decomposed by the excess acid. A section treated with this reagent will reveal specifically only chloride. The second reagent precipitates approximately the same chloride concentration that is affected by the preceding acidified reagent, and in addition maximal concentrations of phosphate and some carbonate. After reduction of the silver precipitates, a section treated with this reagent will usually be darker than a similar section treated with the second reagent; the difference represents phosphate and carbonate present in the section, since approximately the same concentration of chloride is present in each.

Sections to be tested for potassium are transferred on the cover slip to a fairly large cold room whose temperature varies from -1° to $+1^{\circ}\text{C}$. during occupancy. The whole procedure is carried out and the sections are observed with instruments and reagents which have remained at these low temperatures continuously for the duration of the experi-

ments. This is an essential element in increasing the sensitivity of the method.

The paraffin is removed from the sections with anhydrous petroleum ether heated on a warm bar. The petroleum ether is burned off as in the test for chloride, and when the cover slip cools to room temperature, the section is covered with one drop of a 12% solution of sodium cobalti-nitrite prepared according to the method of Bilmann (Treadwell and Hall, '16). The reagent is drained off directly and replaced by glycerine. The cover slip with its section is mounted on a slide, section side down.

Observed with the microscope, potassium appears as crystals of sodium potassium cobalti-nitrite. With the oil immersion lens, these crystals are just visible as brilliant yellow short rods with rounded ends against a pale yellow diffuse background. The precaution to maintain reagents, cover slips, sections, slides, instruments and microscope all at close to 0°C. was taken because the crystals are exceedingly insoluble at this low temperature, though they are quite soluble at ordinary room temperatures. A thin commercial mineral oil was used instead of cedar oil because the latter became too viscous for use with the microscope in the cold room. The petroleum ether was warmed on a hot plate in order to shorten the time required to dissolve the paraffin from the sections.

RESULTS

Chloride in frog sartorius muscle as determined with the use of acidified silver nitrate almost saturated with silver chloride (reagent 1). In all the muscles examined histochemically, no chloride could be observed in the muscle cell. All the chloride visualized by this method was present in the extracellular phase, the tissue fluid spaces of the reticular and collagenous connective tissues. Differences in the chloride concentration of muscles subjected to different experimental conditions (as determined quantitatively by Fenn and co-workers) were manifest only as differences in the amount of chloride visible in the interstitial connective tissue.

In sections of muscles carefully dissected and frozen immediately on removal, the connective tissue appeared as a very pale yellow to a frank yellow in contrast with the complete absence of color in the muscle fibers. Viewed through the dark-field condenser, or by direct transillumination, this color was resolved with high power lenses into a variable number of minute, scattered, black or brown granules distributed evenly outside the muscle walls in the connective tissue. The quantitative variations in the choride concentration of normal muscles is matched by the variable number of black granules visible in sections of the same thickness from different muscles. In some, the reduced silver was very scarce, while in others the particles were closely spaced.

When the chloride concentration in this muscle was decreased by its removal and immersion in 4.5% glucose at 22°C. for 3 to 5 hours, generally no chloride was visible on sectioning. Occasionally sections from some muscles treated in this way showed the minimum number of granules which were visible in the normal, untreated muscle.

When the muscle chloride concentration was increased, the only change visible on sectioning was a consistent, increased amount of chloride visible in the intercellular connective tissue; no chloride appeared in the muscle cell except occasionally in a fiber at the periphery which may have been damaged in the manipulations. The chloride concentration was increased in several ways: 1) by immersion of the sartorius muscle for 3 to 5 hours at 22°C. in isotonic Ringer's solution, 2) by a similar immersion in Ringer's solution containing twice the usual concentration of solutes, 3) by the intraperitoneal injection into the live frog of 1 cc. of 10% NaCl 15 minutes before removal of the muscle, 4) by stimulation of the muscle of a urethanized frog for 30 minutes preceding its removal. The last was accomplished by stimulation of the thigh with a 60 cycle A.C. current at 1.5 to 2.5 volts by means of a copper plaque sewed to the skin over the urostyle and a platinum wire passing through the gastrocnemius muscle and wound around the skin. The current was con-

tinuous in some cases and interrupted in others at the rate of 30 to 40 per minute.

The color range of the connective tissue in sections of muscles treated as described in the preceding paragraph varied from the frank yellow of the uppermost normal range to brown or black. These resolved themselves into minute black granules of variable density; rather closely spaced in sections of some muscles, forming a dense veil in the reticulum surrounding the uncut surface of the muscle fiber in others, with still others showing intermediate stages. In general, the order of increasing density of reduced silver particles visible was 1) muscles of injected frogs, 2) stimulated muscles, 3) muscles immersed in Ringer's solution, and 4) muscles immersed in 2 X Ringer's solution. In all muscles, whether experimentally manipulated or normal, there was more visible chloride in the collagenous connective tissue than in the reticular connective tissue.

Phosphate-carbonate in frog sartorius muscle determined as the difference between the color intensities produced by a) acidified silver nitrate almost saturated with silver chloride (reagent 1) and b) silver nitrate almost saturated with silver chloride and silver phosphate (reagent 2). In all muscles examined histochemically phosphate-carbonate was observed in the muscle cell and in the connective tissue surrounding it. Differences in the intracellular distribution of these ions were too vague and uncertain to be clearly visible in sections of muscles from normal or injected frogs or of muscles immersed in Ringer's or in 2 X Ringer's solution. The extracellular differences were somewhat more marked but also uncertain.

When treated with reagent 2 the cytoplasm of the muscle cells took on a yellowish color which was uniform throughout the cell as compared with the colorless appearance of the cytoplasm after the application of reagent 1. This indicates a uniform cytoplasmic distribution of phosphate-carbonate which is quite at variance with the linear arrangement of these ions described by earlier histochemists and deduced from recent microincineration studies.

The color intensity of all cells in a section was of the same order except for an occasional darker cell on the periphery which may have been injured during its removal.

The intercellular connective tissue always appeared darker with reagent 2 as compared with reagent 1. It appeared that the added increment of color was greater in muscles immersed in Ringer's solution than in normal muscles. The color differential which indicates the presence of phosphate-carbonate was greater in the collagenous connective tissue than in the reticular connective tissue, indicating the presence of a greater amount of these ions in the former.

Potassium in frog sartorius muscle determined with sodium cobalti-nitrite at a low temperature. The normal frog sartorius muscles used for the study of the localization of chloride and phosphate-carbonate were also examined for the distribution of potassium. The crystals representing potassium pack the whole cell cytoplasm uniformly. There is a certain variability in the density of the crystals which one would expect in view of the individual differences Fenn and his co-workers found in such muscles in his quantitative analyses. But the variability is within a certain recognizable range. This uniform distribution was absent only when a known source of error was introduced, as by the addition of slight traces of water to the section by condensation, or by warming the slide or cover slip. In these cases, the crystals were very much larger, and were sharply limited to the dark band. This artifactual distribution of potassium within the muscle cell corresponds with that given by Macallum ('08) and also with that which one would expect from the sharp localization of mineral ash in the dark line after microincineration. A few crystals which were generally larger were scattered in the collagenous and reticular connective tissue spaces. They were more numerous in the former. The distribution of potassium in resting muscles from injected frogs, in stimulated muscles or in muscles immersed in either of the Ringer solutions showed no great differences from that in normal muscles.

This may be due to the fact that the crystals in the sections are so numerous that a slight increment or decrement would be undetectable.

Incidentally, the distribution of potassium in the nucleated red blood corpuscles present in the sections may be noted. Numerous very tiny yellow crystals of sodium potassium cobalti-nitrate packed the cytoplasm surrounding the clear oval nucleus which was entirely devoid of them.

DISCUSSION

A true understanding of the results described in the preceding section is naturally conditional on an appreciation of the nature and limitations of the methods employed. Hence, these subjects will be extensively discussed as a prelude to a discussion of the distribution of the ions in skeletal muscle.

Discussion of methods. Numerous controls extending over a long period of time have convinced the writer that with proper precautions there is no significant detectable diffusion of chloride, phosphate, carbonate or potassium, and that the observed distribution of these substances in cells and tissue fluids actually does represent essentially their real localization. Reasons are also given in this discussion of the methods for believing that the methods are also quite sensitive and specific. There is, in the final analysis, no complete proof that the distribution of ions described in this paper actually represents their real localization in the living cells. This fundamental difficulty occurs not only in the particular problem under investigation, but is quite general in the biological sciences. There are two principal means of circumventing this problem. The first is to show that when certain precautions are not taken, certain errors are introduced. In other words, the procedure indicated is to introduce certain known errors in technique (as, for example, the addition of water to anhydrous petroleum ether used in this procedure) and then observe the difference in the distribution of chloride or phosphate-carbonate. That difference is then the result of the diffusion of these ions caused by the introduction of water in the reagent

used. In the same way, each step in the procedure must be controlled. Finally when every step is controlled in this way, the assumption is that the precautions taken in the procedure are adequate, and that the distribution of the substances studied represents approximately their real distribution. It follows, then, that subsequent refinements of technique that have escaped the writer up to now will improve the method further. In this sense, the procedure outlined in this paper will serve as at least a beginning toward the elaboration and development of a useful, accurate technique for the detection of these various ions. A second way of determining the adequacy of the histochemical tests is to compare the results obtained by means of them with those yielded by other techniques—in this particular case, with the theoretical conclusions from chemical analyses of skeletal muscle. As the following paragraphs will emphasize, such a comparison shows that there is a striking agreement in the composition of the intra- and extracellular phases of skeletal muscle with respect to chloride, phosphate-carbonate and potassium as determined histochemically by the methods described in this paper and by chemical analyses of whole muscle.

The objects attained by the procedures described in the section on Methods are: 1) limitation of the diffusion of chloride, phosphate, carbonate, and potassium to the point where it becomes insignificant, 2) a greater sensitivity of the reactions so that minimal concentrations of these ions can be detected. The steps in the procedure that limit the diffusion of the ions that are being studied are: a) almost instantaneous freezing in liquid air and removal of water present in the tissues at low temperatures, b) direct infiltration of the dried tissue in paraffin freed from water and low boiling point oils by heating in a vacuum above the boiling point of water, c) absence of an affixative (Mayer's albumen) containing water, which if present at a later point, causes diffusion of salts, d) use of an anhydrous reagent (petroleum ether) to remove the paraffin, e) burning off the petroleum ether instead of slow evaporation, and the use of a low-boiling fraction. During the burning process, the temperature of the

cover slip (and the section) is raised above that of the surroundings thus preventing a condensation of atmospheric water on the section, such as takes place during slow evaporation from the cover slip due to the cooling effect.

The steps taken in the procedure to assure great sensitivity of the reactions for chloride, and phosphate-carbonate all follow from solubility product relations. They are: a) the very high concentration of silver ion in the reagent, b) the use of a minimal volume of water in the reactions, c) the addition of silver chloride and silver phosphate to a point just short of saturation (as a precaution against evaporation and precipitation of excess silver chloride and phosphate on the section from the reagent preceding the application of glycerine) in the reagents used to detect chloride and phosphate-carbonate, respectively, d) the substitution of light to reduce the precipitated silver chloride, phosphate and carbonate instead of the customary reducing solutions.

The sensitivity of the reaction for potassium is enhanced by: a) use of a concentrated reagent, b) use of a minimal amount of water, c) conduction of the test and observation of the sections in a cold room with reagents and instruments all at the same uniformly low temperature.

In spite of the precautions taken to increase the sensitivity of the methods, there is necessarily an irreducible amount of these ions in sections which escape detection. Hence conclusions drawn from histochemical studies with these methods apply definitely only to ion concentrations above a certain limited but not definitely known range; below this the methods are entirely inoperable.

The reagents used appear to be specific for the identification of the ions which they are designed to demonstrate. The great danger in the use of silver salts for the demonstration of chloride and phosphate-carbonate is the non-specific confusing reduction of silver by the reducing substances common to all tissues and by the slow action of light on silver-protein compounds. Sections tested as usual for chloride and phosphate-carbonate, but kept in the dark for 30 minutes without

exposure to light show no trace of reduced silver at the end of that time. After exposure to a bright light for a short time or to daylight for a longer time, sections such as these, or others which have been washed with 1 to 5 cc. of distilled water preceding or following the application of the silver reagents, show a slow progressive reduction of silver by the tissue proteins. This is manifested by a uniform brown tint becoming more pronounced in time. This phenomenon is completely absent in sections observed directly after reduction by light as described, or for a short time thereafter. When the proper precautions are taken there is a wide safety factor between the time required to reduce the chloride, phosphate, carbonate and hydroxide precipitates of silver and the time at which the tissue proteins begin to be reduced. The reduced silver observed in sections should represent specifically chloride alone or together with phosphate and carbonate, depending on the reagent used. A study of the chemical analyses of various structures makes it probable also that none of the substances which interfere with the specificity of the potassium reaction (ammonium, the alkaline earth metals, lithium, or sodium) are present in sufficiently large amounts in skeletal muscle to interfere with the specificity of the reagent for potassium. All the evidence leads to the conclusion that the reagents used as prescribed are specific for the ions they are designed to demonstrate in the structures to which they have been applied.

It will be noted that it is advisable to dehydrate tissues at -60°C . or -62°C . if they are to be used for histochemical studies for these ions. This temperature was recommended several years ago by Scott ('33) and more recently by Hoerr ('36). In the course of this investigation there was developed good evidence to show that under certain conditions in skeletal muscle there is some diffusion of chloride and phosphate carbonate during the process of drying if the tissue is dehydrated at -30°C . This becomes markedly pronounced if the tissues are first kept at -30°C . for a month and then dried in a vacuum at the same temperature. The behavior of

these substances, however, is in marked contrast with that of ferrocyanide in the rabbit kidney and of calcium phosphate in liver, spleen, bone marrow and lymph node. An exhaustive series of controls demonstrate that ferrocyanide in the mammalian kidney has an identical distribution in sections of material dried at -60°C . and at -30°C ., within the limits of the method employed to demonstrate this substance. In the same way, no evidence could be uncovered of the diffusion of calcium phosphate ingested by phagocytes. The stability of ferrocyanide during dehydration may be related to its high eutectic point or to some other factor. The indiffusibility of calcium phosphate during dehydration may be referable to its high eutectic point; it is certainly related to its marked insolubility in water solutions. The behavior of calcium phosphate in the rat and dog will be described in two forthcoming papers.

The methods described in this section suffer from several possible sources of error. Although they have been utilized successfully in several specific problems, the procedures cannot be applied universally and indiscriminately to a variety of organs and processes. During the mounting of sections on the cover slip, when the paraffin is being melted and pressed down again, and again after the paraffin is dissolved by the petroleum ether, there is a shifting of the paraffin and of the section; together with it there may be a passive movement of inorganic and organic, free or attached particles. This may result in a purely mechanical shifting of the inorganic constituents that destroys their strict localization.

It must be emphasized at this point that whereas the tests for chloride and phosphate-carbonate are specific under certain conditions, an evaluation of the total quantity of the latter depends on the method of difference. That is, the total quantity of phosphate-carbonate is indicated by the difference in color intensity and number of granules between a section demonstrating phosphate, carbonate and chloride, and another showing only the same amount of chloride. When phosphate-carbonate is small in amount, or when the chloride content is high, an error in interpretation may be introduced.

The amounts of chloride, phosphate, carbonate and potassium present in each section are so minute, and their diffusibility so great, that the admission of small traces of water is capable of disturbing the strict localization so essential for histochemical work. Attempts have been made throughout to avoid such pitfalls and to accept for study only those sections which have been carried through the procedure under the most favorable conditions.

Finally, another possible source of error in histochemical investigations of inorganic substances has been emphasized by Hoerr, who pointed out that proteins contain a certain residuum of water which cannot be removed in a vacuum even at temperatures higher than those customarily used. The extreme measures which must be taken to deprive the proteins of this residuum would suggest that the water may be 'bound' by capillarity, or that it may be an essential part of the organic and inorganic molecular structure, or that it may be included in some other way so as to be not available as water of solution and subsequently be responsible for diffusion of solutes. If this is so, then the water remaining in tissues after they have been dehydrated in the vacuum is of no immediate concern in histochemistry. The following experiment proves that this is in fact the case. Glass tubes with an inner diameter of 5 mm. were closed at one end with DeKhotinsky cement. Through the open end, serum was added to a height of about 1 cm. They were frozen by immersion in liquid air. The tubes were removed to the air for a moment and covered with an equally high layer of serum in which crystalline sodium ferrocyanide or sodium thiocyanate had been dissolved. The tubes were again immersed in liquid air, and after freezing they were transferred to a vacuum chamber and dried at -30°C . After drying, several tubes were kept in a vacuum desiccator at -30°C . and at room temperature, in a lightly stoppered container at -30°C . and at room temperature. Immediately after drying, and at intervals thereafter up to 3 months from the time of dehydration, one of each set of tubes was removed, and brought to room temperature.

The de Khotinsky seal was carefully removed from the bottom of the column of dried serum. The solid serum was then chipped away, without disturbing the column above, up to a point about 2 mm. from the ferrocyanide or thiocyanate level. No trace of these salts could be detected under these conditions in any fraction of the lower level of ferrocyanide-free serum. At the end of 3 months, the tubes still contained about 0.6% of water as determined by the conventional method of heating a weighed sample.

Because it is intimately related to the nature of the methods employed it is desirable at this point to insert a general statement regarding the interpretation of the findings. The methods for the demonstration of chloride and potassium as used in the present investigation visualize the total amount of these substances present in a microscopic section of definite thickness. In two specimens of equal thickness an increased amount of chloride in one may indicate one of two things: 1) An increase in the amount of fluid containing chloride with no change in its concentration. For example, when considering the reticular connective tissue, if the amount of reticular connective tissue (+ tissue fluid) is doubled and the concentration of chloride in the tissue fluid remains the same, then more chloride will be visible in the sections of the dried material. 2) An increase in the concentration of chloride in the fluid with no change in the volume of the tissue fluid. Again considering the reticular connective tissue, if the volume of reticular tissue (+ tissue fluid) remains the same, but the concentration of chloride in the tissue fluid is doubled, then more chloride will be observed with the microscope in sections of the dried material. Variations in the observed amount of chloride in the preparations, then, may indicate differences in the amount of chloride-containing fluid, or in the concentration of chloride in the fluid, depending on the amount of fluid present. The amount of free water present in cells and intercellular material cannot be determined by the method in use. However, in the absence of definite knowledge on the subject, there is a great body of knowledge derived from other sources which can be applied to certain aspects of the problem.

Attempts will be made in this paper to interpret variations in the total amount of chloride as observed in sections with differences in the amount of chloride-containing fluid and with fluctuations in the concentration of chloride in the fluid.

Discussion of results. The results described in the preceding section serve as an actual demonstration of what had been assumed or deduced regarding the intracellular distribution of these four ions in striated muscle fibers. Potassium is present in very large amounts in the cytoplasm, phosphate-carbonate is present in moderate amounts, and chloride is completely absent. Assuming this complete absence of chloride from the cytoplasm, biochemists and physiologists made quantitative estimates of the inorganic intracellular composition of muscle fibers (Fenn). Accordingly, it is believed that the frog sartorius muscle cells contain potassium, sodium, magnesium, calcium, bicarbonate and phosphate. The substantiation by direct histochemical methods of this basic assumption lends greater force to the subsequent derived composition of muscle cells as given above.

Potassium and phosphate carbonate are represented as being uniformly distributed in the muscle fiber, with no apparent relation to its longitudinal or transverse striations. This is again in agreement with the conclusion to which Fenn was led: that "the distribution of potassium in the normal untreated cell is (not) essentially different from the distribution of water." Such findings are in conflict with the sharply localized distribution of ash in the dark bands after micro-incineration and with the earlier obviously faulty histochemical methods.

The muscle chloride, then, was found to be confined entirely, under all the conditions of this investigation, to the reticular and collagenous connective tissue framework, dissolved in the tissue fluid which infiltrates and permeates the interstitial tissue. This distribution was assumed by Fenn and his co-workers, but its demonstration gives greater credence to the calculations of Fenn and co-workers for the frog sartorius muscle and of Hastings and Eichelberger ('37) for mammalian muscle of the proportion of a skeletal muscle which

is not occupied by muscle fibers but rather is given over to the intercellular, tissue-fluid-containing spaces. The tissue fluid spaces occupy a calculated volume of about 14% in the normal frog sartorius muscle, and about 18% in the rectus abdominus muscle of the dog.

In addition to chloride, the tissue fluid contains visibly demonstrable phosphate-carbonate and potassium. All four ions were present in greater amounts in the collagenous tissue than in the reticular connective tissue. This is probably referable to the fact that in sections of striated muscle there are no large areas of reticular fibers, comparable to those occupied by collagenous fibers. The greater amount of these ions in the latter as observed in sections, then, may reflect only differences in the amount of tissue fluid present in different volumes of connective tissue.

The observed increased amount of chloride in the tissue fluid spaces of muscles stimulated indirectly is again a confirmation of the conclusions drawn from quantitative analyses of whole muscle. It is to be correlated with the increase in the tissue fluid and the lymph flow which is known to take place in contracting muscles.

SUMMARY AND CONCLUSIONS

1. Methods are described for the histochemical study of chloride, phosphate-carbonate, and potassium in certain structures.

2. These methods were utilized in a study of the distribution of these ions in the sartorius muscle of the frog. Phosphate-carbonate and potassium, but no chloride was detected in the muscle fiber. All four ions were present in the extracellular tissue fluid spaces.

3. These results are on very close agreement with certain conclusions drawn recently from chemical analyses of whole muscle.

LITERATURE CITED

- FENN, W. O. 1936 Electrolytes in muscle. *Physiol. Rev.*, vol. 16, pp. 450-487.
- FENN, W. O., AND D. M. COBB 1934 The potassium equilibrium in muscle. *J. Gen. Physiol.*, vol. 17, pp. 629-656.
- 1936 Electrolyte changes in muscle during activity. *Am. J. Physiol.*, vol. 115, pp. 345-356.
- FENN, W. O., D. M. COBB AND B. S. MARSH 1934 Sodium and chloride in frog muscle. *Am. J. Physiol.*, vol. 110, pp. 261-272.
- GERSH, I. 1938 The fate of colloidal calcium phosphate in the dog. *Am. J. Physiol.* (In press.)
- 1938 Histochemical studies on the fate of colloidal calcium phosphate in the rat. *Anat. Rec.*, vol. 70, pp. 331-349.
- HASTINGS, A. B., AND L. EICHELBARGER 1937 The exchange of salt and water between muscle and blood. I. The effect of an increase in the total body water produced by the intravenous injection of isotonic salt solutions. *J. Biol. Chem.*, vol. 117, pp. 73-93.
- HOERR, N. L. 1936 Cytological studies by the Altmann-Gersh freezing-drying method. I. Recent advances in the technique. *Anat. Rec.*, vol. 65, pp. 293-317.
- LISON, L. 1936 *Histochemie Animale. Méthodes et Problèmes.* Paris: Gauthiers-Villars.
- 1936 Recherches histochimiques sur la sécrétion chlorhydrique de l'estomac. *Zeit. f. Zellf. u. mikr. Anat.*, Bd. 25, S. 143-159.
- MACALLUM, A. B. 1908 *Die Methoden und Ergebnisse der Mikrochemie in der biologischen Forschung.* *Ergeb. d. Physiol.*, Bd. 7, S. 552-652.
- SCOTT, H. G. 1933 A critical study and review of the method of micro-incineration. *Protoplasma*, vol. 20, pp. 133-151.
- TREADWELL, F. P. 1916 (Translated by W. T. Hall.) *Analytical Chemistry.* Vol. 1, 4th English edition, p. 81. New York. John Wiley & Sons, Inc.

HISTOCHEMICAL STUDIES ON THE FATE OF COLLOIDAL CALCIUM PHOSPHATE IN THE RAT

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ONE PLATE (FOUR FIGURES)

INTRODUCTION

Within recent years the metabolism of calcium and phosphorus has been studied intensively, particularly in blood. In whole blood, calcium is distributed entirely or almost entirely in the plasma; the blood cells contain little or no calcium. In the plasma 40 to 60% of the calcium is diffusible or is capable of passing readily through membranes impermeable to colloids, the remaining portion being held back with the proteins. The diffusible calcium is almost completely ionized in contrast to the non-diffusible calcium, which is bound to the plasma proteins either as an alkali proteinate or as part of a negatively charged protein ion. In either case, the bound calcium is in equilibrium relations with the ionized diffusible calcium.

The inorganic phosphorus of the blood is distributed more or less equally between the blood cells and the plasma. In the latter, under normal conditions, the phosphate is said to be nearly entirely diffusible in vitro, though in vivodialysis experiments 85% of the inorganic phosphorus is diffusible.

When calcium salts are introduced intravenously in the organism, the concentration of diffusible inorganic phosphate in the plasma falls below the normal level; when inorganic phosphates are injected intravenously in the organism, the blood calcium falls below the normal level. There appears to be an inverse relationship under these conditions between the diffusible plasma calcium and phosphate.

This inverse relationship can be understood only by a consideration of the physical states of these substances in the blood. When the calcium ion concentration is raised sufficiently as by intravenous injection, the product of the concentration of the calcium and phosphate ions exceeds the solubility product of calcium phosphate, and thus a non-diffusible, unionized, colloidal substance of low solubility is formed. The nature of this compound is not certain—it appears to be CaHPO_4 or $\text{Ca}_3(\text{PO}_4)_2$. In the same way, the equilibrium reaction is disturbed and driven toward completion by the intravenous administration of soluble phosphate in a normal animal. In the first case, as the colloidal material is formed, the phosphate concentration in the plasma is depressed; in the second case, the calcium concentration in the plasma is depressed. There is good evidence from a variety of sources that under certain conditions this colloidal non-diffusible calcium-phosphate complex is actually formed both in vitro and in vivo and that it does adequately explain the observed inverse relationship of diffusible calcium and phosphate under these conditions.

One would expect that this colloidal calcium phosphate could not circulate long in the blood stream without being phagocytized by the macrophages of the liver, spleen and bone marrow. In this paper the course of phagocytosis is described after the intravenous administration of calcium and phosphate salts, and of preformed colloidal calcium phosphate injected as such. The deviation from the normal of the process of phagocytosis of colloidal calcium phosphate is outlined under conditions which are expected to alter the calcium or phosphate ion concentration of the plasma. These conditions are the injection of parathyroid extract and the administration of citrate.

The appropriate administration of parathyroid extract is known to increase the calcium ion concentration in the blood plasma, without affecting markedly the phosphate ion concentration. The addition of citrate to plasma results in the formation of a negatively charged metal-containing ion which

is in equilibrium relations with the calcium ions. The net result of the administration of citrate to an animal is hence a reduction of the plasma calcium ion concentration. The effect of the increase or decrease in the calcium ion concentration of the plasma effected in these ways has been studied in relation to the course of phagocytosis of colloidal calcium phosphate.

Unfortunately no chemical analyses of blood or tissues were made coincidentally with the histochemical studies. This obvious defect is not actually a very great handicap. For, in general, procedures were used which have been studied chemically so many times that their results are in a qualitative sense clear-cut. A second series of experiments performed on dogs are reported in another journal. They are more closely correlated with chemical studies made independently by McLean and Hinrichs ('38).

MATERIAL AND METHODS

The experiments described in this paper were performed on 130 adult rats from different sources. The majority of the rats were maintained for at least 2 weeks on dried cubes sold as dog food and found to be highly satisfactory in the breeding of rats in the animal quarters here. In one experiment the diet consisted of dried milk with added salts, with the end in view of varying the Ca:P ratio within wide limits. The use of these diets was described by Shelling and Josephs ('34).

Unanesthetized rats were injected through the greater saphenous vein with calcium and phosphate salts. Unless otherwise indicated, the calcium was injected as a 5% solution of calcium chloride ($\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$); where noted in some experiments, it was administered in 2% solution. The phosphate injected was a 5% solution of anhydrous dibasic sodium phosphate brought close to the neutral point by the addition of a 5% solution of monobasic sodium phosphate. These salts were injected in amounts depending on the body weight; the quantities administered are given in the tables as Ca and P present in the solution.

Colloidal calcium phosphate suspended in horse serum was prepared by a very convenient method described by McLean in a personal communication. The colloidal calcium phosphate formed by this procedure tends to settle out on standing; hence no colloidal suspension was used for injection purposes which had stood for more than 30 minutes in the incubator. In the dosage in which all these substances were administered they were apparently not toxic.

The parathyroid extract was kindly supplied by Eli Lilly and Co., to whom thanks are due.

The animals were killed by a blow on the head (and generally bled at the same time) at definite time intervals after the end of the injection. Pieces of liver, spleen, lymph node, and bone marrow were removed in that order as rapidly as possible and frozen in liquid air. They were dried in a vacuum at $-30^{\circ}\text{C}.$ and then infiltrated with Grüber's paraffin (m.p. 50° to $52^{\circ}\text{C}.$) for 15 minutes. Just before use, the paraffin was heated in a vacuum at $100^{\circ}\text{C}.$ or higher for 15 minutes. Sections 15μ thick were cut and mounted on large cover slips. The histochemical method of testing these sections for their total phosphate and carbonate content has already been described in the preceding paper of this volume. However, in the experiments reported in this paper, there is every reason to believe that the substance precipitated by the reagent and observed as black or brown granules in the phagocytic cells is actually phosphate, or at least preponderantly so. With this reservation, then, the precipitate will be referred to, hereafter, as simply phosphate.

It was found that when colloidal calcium phosphate has been taken up from the blood stream by phagocytes it appears after the use of the second reagent as brown or black granules of a size and number which cannot be confused with the vague homogeneous extremely pale coloring that may be present in cells or connective tissues not containing the colloidal compound. This vague coloring may represent cytoplasmic or tissue fluid chloride and phosphate-carbonate. The presence and appearance of colloidal calcium phosphate in phagocytes

is so unmistakable that in most cases, only reagent 2 was used. In studying lymph nodes and sometimes spleens, however, black phagocytized particles may be normally present in some cells. Hence sections of lymph node treated with the chloride reagent (reagent 1) were always used as a control. In doubtful cases, sections of the spleen similarly treated were used for comparison.

All sections were examined for phosphate. Those which were particularly favorable were also tested for calcium. For this latter element the reagent consists of a saturated aqueous solution of sodium alizarine sulfonate diluted with water (2 or 3 drops of water to 10 cc. of reagent). The stain was applied to sections of the liver after the petroleum ether was burned off as in the test for phosphate. When the section approached room temperature, it was covered by a drop of the reagent which was decanted in 30 seconds and replaced by a drop of chemically pure glycerine. Calcium appears as an orange color which is not as discretely separated into granules as after the application of the silver reagent. Moreover the granules are less numerous with this calcium test than after the phosphate reagent is applied. Whenever used, the localization of the calcium and phosphate in the Kupffer cells was so nearly identical that it was thought justifiable to regard the granules of phosphate made visible in the cytoplasm of phagocytic cells as colloidal calcium phosphate. This assumption is justified also by the greater number of recent chemical studies of calcium and phosphate in the blood.

RESULTS

I. Effect of diet on the phagocytosis of calcium phosphate (table 1)

In spite of the fact that the amount of phosphorus and calcium in the diets varied widely, there is no phagocytosis of colloidal calcium in the liver of normal rats when they are not injected intravenously with calcium. The fluctuation in the plasma calcium and phosphate ion concentrations effected by these various diets, though appreciable, are of such small

magnitude that apparently no (or not enough) colloidal calcium phosphate exists as such in the circulating blood.

After the subminimal intravenous administration of calcium ions, there is no phagocytosis of calcium phosphate in the liver of rats 5 and 10. Apparently in these cases also, the product of the concentration of calcium and phosphate does

TABLE 1

RAT NO.	DIET	PER CENT Ca IN DIET	PER CENT P IN DIET	RATIO Ca: P IN DIET	DOSAGE OF Ca IN MILLIGRAMS PER 100 GM. BODY WEIGHT	BODY WEIGHT IN GRAMS	TIME IN MINUTES	DEGREE OF PHAGOCYTOSIS IN LIVER
1, 2, 3, 4	Optimal Ca, P	0.51	0.50	1: 1				0
5	Optimal Ca, P	0.51	0.50	1: 1	1.9	235	10	0
6, 7, 8, 9	High Ca, low P	1.87	0.2	9.4: 1				0
10	High Ca, low P	1.87	0.2	9.4: 1	2.3	185	10	0
11, 12, 13, 14	Low Ca, low P	0.27	0.2	1.4: 1				
15	Low Ca, low P	0.27	0.2	1.4: 1	2.1	225	15	+
16, 17	Low Ca, high P	0.27	0.85	0.32: 1				0
18	Low Ca, high P	0.27	0.85	0.32: 1	2.3	195	10	+++
19, 20, 21, 22	High Ca, high P	1.87	0.85	2.2: 1				0
23	High Ca, high P	1.87	0.85	2.2: 1	2.3	200	10	++

Table 1 demonstrates the influence of diet on the phagocytosis of calcium phosphate by the Kupffer cells of the liver following the intravenous injection of calcium chloride. All rats were kept on the diets recommended by Shelling and Josephs, slightly modified, for 2 weeks prior to the experiment. There was no evidence of phagocytosis of calcium phosphate in the spleen, bone marrow or lymph node in these animals. The term degree of phagocytosis is used to designate an estimate of the number of actively phagocytic cells and the size and number of granules ingested by these cells.

not exceed or exceeds only slightly the solubility product of calcium phosphate. The liver of the other animals injected intravenously with calcium under similar conditions shows various degrees of phagocytosis of colloidal calcium phosphate. In these cases, the initial concentrations of the plasma calcium or phosphate or both are probably greater than normal and the addition of calcium to the blood results in the formation

of appreciable amounts of colloidal calcium phosphate. This is then phagocytized.

The explanation offered here for the effect of diet on the phagocytosis of calcium phosphate is tentative and of course must await confirmation by a chemical analysis of the blood plasma of rats treated as in this experiment. No matter what the final interpretation the experiment demonstrates beyond doubt that the calcium and phosphate content of the diet of rats clearly influences the formation and phagocytosis of colloidal calcium phosphate after the intravenous injection of calcium. For this reason, in all subsequent series, all the animals used in any one experiment were fed with the same diet for a longer or shorter time which was never less than 2 weeks.

TABLE 2

RAT NO.	DOSAGE OF P IN MILLIGRAMS PER 100 GM. BODY WEIGHT	BODY WEIGHT IN GRAMS	TIME IN MINUTES	DEGREE OF PHAGO- CYTOSIS IN LIVER
24	2.0	140	15	+
25	2.0	170	30	+
26	3.0	270	15	++
27	3.0	320	30	+
28	4.0	240	15	++++
29	4.0	180	30	++++

Table 2 demonstrates the increasing phagocytosis of calcium phosphate by the Kupffer cells of the liver following the intravenous injection of increasing doses of a neutral sodium phosphate solution.

II. The effect of varying the amount of colloidal calcium phosphate in the blood stream on the degree of phagocytosis of this compound

In these experiments, soluble phosphorus and calcium salts are injected intravenously in amounts small enough to result in a just appreciable formation and phagocytosis of colloidal calcium phosphate. The increase in the plasma concentration of either one of these ions effected in this way is apparently great enough to result in the formation and phagocytosis of the colloidal calcium salt. The amount of the insoluble compound formed in the circulating plasma is increased in the

same way by injecting greater amounts of calcium chloride (table 3) or sodium phosphate (table 2), by injecting the small amount of both salts successively in the same animal (table 4), or by injecting preformed colloidal calcium phosphate after the previous administration of a calcium salt

TABLE 3

RAT NO.	DOSAGE OF Ca IN MILLIGRAMS PER 100 GM. BODY WEIGHT	BODY WEIGHT IN GRAMS	TIME IN MINUTES	DEGREE OF PHAGOCYTOSIS IN	
				Liver	Spleen
30	1.3	330	15	++	0
31	1.3	320	30	0	0
32	2.7	210	15	++	+
33	2.7	370	30	+	tr.

Table 3 demonstrates the increasing phagocytosis of calcium phosphate by the phagocytes of the liver and spleen, following the intravenous injection of increasing doses of calcium chloride. The salt was administered as 2% solution in rats 30 and 31, and as 5% solution in rats 32 and 33.

TABLE 4

RAT NO.	DOSAGE OF Ca AND P IN MILLIGRAMS PER 100 GM. BODY WEIGHT	BODY WEIGHT IN GRAMS	TIME IN MINUTES	DEGREE OF PHAGOCYTOSIS IN	
				Liver	Spleen
34	2.5 P	250	15	0	0
35	2.5 P	200	30	+	0
36	2.7 Ca	220	15	++	+
37	2.7 Ca	370	30	+(+)	tr
38	2.7 Ca 2.5 P	150	15	++++	+
39	2.7 Ca 2.5 P	400	30	+++	+++
40	2.7 Ca 2.5 P	210	60	+++	++

Table 4 demonstrates the increased phagocytosis of calcium phosphate by the phagocytes of the liver and spleen following the intravenous administration of calcium chloride and sodium phosphate injected successively, as compared with the less marked phagocytosis resulting from the intravenous injection of equivalent doses of either calcium or phosphate alone. In the double injections, the phosphate solution was injected directly after the previous administration of the calcium salt.

(table 5). In all cases, the number of colloidal particles in the circulating plasma is magnified. In acute experiments such as those described here, the number of phagocytes lining the blood stream and the rate of blood flow is a relatively

constant factor. Hence, as the number of colloidal particles in the circulating plasma is increased, the number of collisions between macrophage and calcium phosphate particles is increased, and the degree of phagocytosis is correspondingly heightened. At the same time, the period during which the granules are retained in the cytoplasm of the phagocytes is also extended. The morphology of phagocytic cells which have ingested colloidal calcium phosphate may be understood by an examination of figures 1 to 4.

TABLE 5

RAT NO.	DOSAGE OF Ca IN MILLIGRAMS AND OF $\text{Ca}_3(\text{PO}_4)_2$ IN CUBIC CENTIMETERS PER 100 GM. BODY WEIGHT	BODY WEIGHT IN GRAMS	TIME IN MINUTES	DEGREE OF PHAGOCYTOSIS IN	
				Liver	Spleen
41	1.4 Ca	330	15	++	0
42	1.4 Ca	310	30	0	0
43	1.4 Ca	320	60	0	0
44	0.7 $\text{Ca}_3(\text{PO}_4)_2$	250	15	0	++
45	0.7 $\text{Ca}_3(\text{PO}_4)_2$	230	30	+(+)	?
46	0.7 $\text{Ca}_3(\text{PO}_4)_2$	320	60	+	0
47	1.4 Ca	180	15	++++	++++
	0.7 $\text{Ca}_3(\text{PO}_4)_2$				
48	1.4 Ca	270	30	+++	++++
	0.7 $\text{Ca}_3(\text{PO}_4)_2$				
49	1.4 Ca	240	60	++	+++(+)
	0.7 $\text{Ca}_3(\text{PO}_4)_2$				

Table 5 demonstrates the increased phagocytosis of calcium phosphate by the phagocytes of the liver and spleen following the intravenous administration of calcium chloride and preformed colloidal calcium phosphate injected successively, as compared with the less marked phagocytosis resulting from the intravenous injection of equivalent doses of each substance alone. In the double injections, the colloidal calcium phosphate was injected directly after the previous administration of the calcium salt. The calcium chloride was injected in 2% solution.

III. Effect of the previous administration of parathyroid extract on the phagocytosis of calcium phosphate (table 6)

The amount of calcium and phosphate was deliberately chosen as a subminimal or minimal dosage of the salt which would almost or just result in the formation in the blood of appreciable amounts of colloidal calcium phosphate in the selected time interval. The intraperitoneal injection of parathyroid extract in proper amounts causes an increase in the

serum calcium ion concentration which is, however, not great or rapid enough to cause the formation of colloidal calcium phosphate in the circulating blood. This increase in the calcium level is probably more marked in the rats injected over a long period of time than in those treated acutely. Together with and superimposed on the high blood calcium ion concentration induced by the parathyroid extract, however, the intravenous administration of subminimal amounts of either calcium or phosphate ions raises the product of the plasma concentrations of these ions to the point where they exceed the solubility product of the insoluble calcium phosphate. Phagocytosis proceeds as a sufficiently large amount of the colloidal compound is formed. Because the plasma

TABLE 6

RAT NO.	INJECTED WITH PARATHYROID EXTRACT	DOSAGE OF Ca AND P IN MILLIGRAMS PER 100 GM. BODY WEIGHT	BODY WEIGHT IN GRAMS	TIME IN MINUTES	DEGREE OF PHAGOCYTOSIS IN	
					Liver	Spleen
50		1.4 Ca	200	15	0	0
51		2.5 P	230	15	0	0
52	Acute		250		0	0
53	Acute		250		0	0
54	Prolonged		190		0	0
55	Prolonged		240		0	0
56	Acute	1.4 Ca	285	15	+	0
57	Prolonged	1.4 Ca	255	15	++	0
58	Acute	2.5 P	250	15	+	0
59	Prolonged	2.5 P	175	15	+++	tr.

Table 6 demonstrates that the effect of the administration of parathyroid extract in rats is to increase the amount of calcium phosphate phagocytized in the liver and spleen above that which takes place in a normal rat, after the intravenous injection of calcium or phosphate. The administration of parathyroid extract alone raises the calcium and perhaps the phosphate level in the blood, but not enough to result in phagocytosis of calcium phosphate; this increased blood calcium and phosphate ion concentration, however, is responsible for the more pronounced phagocytosis following the intravenous injection of calcium or phosphate as compared to the normal rat in much the same way as is described in connection with table 4. The parathyroid extract was administered as follows: acute, five injections of 200 units each intraperitoneally every hour (total 1000 units), animal used 24 hours after first injection; prolonged, two injections of 200 units each intraperitoneally every 12 hours, four injections of 100 units each every 12 hours (total 800 units), animal used 72 hours after first injection. The calcium was injected as a 2% solution of calcium chloride.

calcium concentration is probably higher in the rats injected over a longer period of time, the amount of colloidal calcium phosphate formed in the plasma and phagocytized by the macrophages is greater. The effect of parathyroid extract on the phagocytosis of calcium phosphate seems to be more simply explained as an effect on the calcium ion concentration than by assuming a specific local action of the active principle present in parathyroid extract on the active phagocytes.

IV. Effect of the intravenous administration of sodium citrate on the phagocytosis of calcium phosphate (table 7)

In the animals into which sodium citrate is injected immediately preceding or following the introduction of colloidal calcium phosphate, it may be assumed for the sake of simplicity that all the substances administered remain in the blood stream and that no particulate material has yet been phagocytized. Under these conditions, the equilibrium reaction briefly discussed in the introduction may be considered to be taking place in the blood plasma even as the compounds are being introduced; that is, the calcium injected as colloidal calcium phosphate is rapidly converted to an unionized more soluble calcium-citrate-ion compound. The result is a great reduction in the number of colloidal calcium particles appearing in the cytoplasm and consequently a corresponding decrease in the degree of phagocytosis of the particulate compound.

In the animal in which sodium citrate is injected after the phagocytosis of calcium phosphate has already taken place, the situation is more complex. The events that take place as the ingested calcium phosphate particles leave the macrophages may take two possible directions.

The first alternative is as follows: The colloidal calcium phosphate enters the cell in granular form and leaves it in the same state. As the number of granules of colloidal phosphate in the plasma is decreased owing to the formation of the soluble calcium-citrate-ion salt, colloidal particles in the cytoplasm of the phagocytic cells leave these cells and enter the

circulating plasma. This description of the events taking place in the organism under these conditions suffers from the disadvantage that macrophages have not been known to liberate their phagocytized particulate matter (such as carbon) except on death.

TABLE 7

RAT NO.	DOSAGE OF $\text{Ca}_3(\text{PO}_4)_2$ AND 10% SODIUM CITRATE IN CUBIC CENTIMETERS PER 100 GM. BODY WEIGHT	WEIGHT IN GRAMS	TIME IN MINUTES	DEGREE OF PHAGOCYTOSIS IN	
				Liver	Spleen
60	1.0 $\text{Ca}_3(\text{PO}_4)_2$	170	5	+++	+++
61	1.0 $\text{Ca}_3(\text{PO}_4)_2$	195	15	++	++
62	1.0 $\text{Ca}_3(\text{PO}_4)_2$ 0.2 citrate	190	5	0	0
63	1.0 $\text{Ca}_3(\text{PO}_4)_2$ 0.2 citrate	175	15	+	0
64	0.2 citrate 1.0 $\text{Ca}_3(\text{PO}_4)_2$	170	5	0	0
65	0.2 citrate 1.0 $\text{Ca}_3(\text{PO}_4)_2$	190	15	+	0
66	1.5 $\text{Ca}_3(\text{PO}_4)_2$ wait 8 minutes 0.2 citrate wait 7 minutes	320	15	0	0
67	1.5 $\text{Ca}_3(\text{PO}_4)_2$	180	8	++	++
68	1.5 $\text{Ca}_3(\text{PO}_4)_2$	210	15	++	+
69	0.2 citrate	250	7	0	0

Table 7 demonstrates that when citrate is injected intravenously just preceding or following the intravenous administration of colloidal calcium phosphate, it prevents the phagocytosis of calcium phosphate which occurs in the absence of citrate. The table also shows that when citrate is injected intravenously in an animal in which active phagocytosis and storage of calcium phosphate has already taken place, the phagocytized calcium is withdrawn from the active cells with a rapidity which far exceeds the normal rate of liberation of the colloidal substance in animals not injected with citrate.

The alternative schema, though more complicated, appears to obviate this difficulty and to have a more general application. The particles of colloidal calcium phosphate introduced into the blood are in equilibrium with the calcium and phosphate ions in the plasma and may be engulfed by the phagocytes with which they may come in contact. This process takes place up to and at the moment when citrate is injected intravenously. At this time the calcium phosphate

particles present in the cytoplasm are converted into calcium and phosphate ions. Both of these ions pass out of the cytoplasm almost as rapidly as they are formed and into the plasma where the ionized calcium is converted to a calcium-citrate complex. This process continues in the presence of excess citrate ions in the plasma until all the solid, particulate calcium phosphate is withdrawn from the macrophages.

This explanation must, of course, remain largely tentative; but in the present state of our knowledge of the physico-chemical state of calcium compounds in blood plasma it appears to account for the observations recorded in this series of experiments quite effectively.

GENERAL DISCUSSION

In the normal animal the blood calcium is confined entirely or almost entirely to the plasma while the inorganic phosphate is distributed more or less evenly between the cellular and fluid portions of the circulating blood. About half of the plasma calcium and the greater part of the plasma phosphate has been shown to be in an ionized diffusible state. When the plasma concentrations of these two active substances are increased to a point where they exceed slightly the solubility product of calcium phosphate, this new insoluble unionized compound is formed in equilibrium relations with both of the ions in solution. It exists in a colloidal state because of the protective action of the plasma proteins. The amount of this colloidal compound existing in the plasma may be varied by influencing one or another factor in this equilibrium reaction. If a minimum amount of colloidal calcium phosphate is formed in blood plasma after the injection of small amounts of an ionized calcium or phosphate solution, the concentration of the particulate compound may be increased in a number of ways: 1) It may be increased by raising the plasma calcium or phosphate ion concentration by means of a) an intravenous injection of a larger amount of either one of these substances, b) an intravenous injection of both substances in minimal amounts, and probably also c) by the

previous administration of parathyroid extract in an appropriate way. 2) It may be increased by injecting intravenously preformed colloidal calcium phosphate suspended in horse serum. If an appreciable amount of colloidal calcium phosphate is already present in the plasma, it may be reduced or even removed entirely by an intravenous injection of sodium citrate. The nature of the reactions resulting in these variations of the amount of colloidal calcium phosphate in the blood plasma is described briefly in the introduction and in the several sections under Results.

It has been demonstrated in supravital studies that the degree of phagocytosis of colloidal particles by macrophages depends on the number of collisions between cell and particle. The degree of phagocytosis as used here is a rather loose term which refers to an estimation of the number of active cells and the number of granules ingested by these cells. In a relatively fixed system such as may be assumed to exist in critical experiments in vivo (in which the number of phagocytic cells and the amount of blood passing them are relatively constant), the greatest variable would appear to be the number of colloidal particles present in the blood stream. If, then, the particulate matter is of the same physical and chemical nature, one would expect that in the organism the degree of phagocytosis would bear a definite relationship to the number of particles present in the blood stream.

These assumptions have been applied and substantiated by the experiments reported in this paper. When the amount of colloidal calcium phosphate in the blood plasma is increased beyond that which is just sufficient to evoke a phagocytic response, the degree of phagocytosis of this compound in the liver and spleen increases correspondingly. These increases were brought about in four different ways, as described in the first paragraph of this section. When the amount of colloidal calcium phosphate in the blood plasma is decreased sharply by the administration of sodium citrate, the degree of phagocytosis of the colloidal compound in the liver and spleen is also decreased.

As long as colloidal calcium phosphate exists in the blood plasma, it is in equilibrium with the calcium and phosphate ions. These ions particularly when they exist in such a high concentration in the blood plasma, are being constantly removed by passing through the kidneys, intestines, and the capillary bed of the body. And as they leave, because of their equilibrium relations, the amount of colloidal calcium phosphate in the plasma is gradually and persistently decreased until none remains in the circulation. During this phase, the results show clearly that the degree of phagocytosis of the colloidal particles is reduced. The granules of calcium phosphate already phagocytized and present in the cytoplasm of the macrophages are reduced in number. The whole process takes place at such a rapid rate that there may be no evidence of phagocytic activity as early as a few minutes to 2 hours after the animal had been injected, depending on the dosage. This occurs even more rapidly when the colloidal calcium phosphate has been suddenly removed from the blood plasma by the administration of citrate ions; the mechanism of this reaction has been discussed at great length under 'Results.'

The phagocytosis of calcium phosphate is most prominent in the liver and spleen. In the experiments performed on rats and dogs there was no indication at any time of phagocytosis of colloidal calcium phosphate in the bone marrow. In some preliminary studies on rabbits injected with rather large amounts of calcium and phosphate salts, there is some evidence that phagocytic activity in the bone marrow may be very marked. In all three species, the lymph nodes were conspicuous in that their free and fixed macrophages never phagocytized the colloidal compound when this was present only in the blood stream even in very large amounts.

The relative ease with which colloidal dyes are engulfed by the macrophages of the liver and spleen, as compared with the relative refractoriness of the phagocytes of bone marrow is well known. The behavior of these structures toward colloidal calcium phosphate is, then, merely a part of the general picture and present no difficult problems of

interpretation. Their different rates of phagocytosis are probably to be correlated with the very marked differences in rate of blood flow, with correspondingly large variations in the number of collisions between particle and macrophage.

The situation is quite different in the lymph node. Here the macrophages are bathed by the lymph derived from the tissue fluid in the area drained by the lymphatics. The only way colloidal calcium phosphate may be formed in the tissue fluid (to be carried by the lymphatics to the lymph nodes) is by a local subcutaneous or intramuscular administration. The elimination into the urine and feces of calcium and phosphate injected intravenously is so efficient that no extreme excess of either ion is permitted to exist in the tissue fluid. Hence the product of the concentrations of these ions extravascularly never exceeds the solubility product of calcium phosphate, and no colloidal granules are formed. There are exceptions to this situation, and these are the sites of normal and pathological calcification. Experiments on dogs are presented in another paper showing that the subcutaneous and intramuscular administration of soluble calcium or phosphate salts or of colloidal calcium phosphate suspended in horse serum do result in a marked phagocytosis of the particulate compound in the lymph node.

SUMMARY

1. Calcium phosphate formed in the circulating blood plasma is phagocytized in the liver, spleen and bone marrow; the lymph node takes no part in this process when the colloidal particles are confined entirely to the blood stream.
2. The degree of phagocytosis may be influenced by variations in the amount of the colloidal compound present in the blood stream.
3. The phagocytized particles are removed from the active cells in a relatively short time; the whole process is quite transitory.
4. The mechanisms involved in these processes are discussed at length.

REFERENCES

- GERSH, I. 1938 Improved histochemical methods for chloride, phosphate-carbonate and potassium applied to skeletal muscle. *Anat. Rec.*, vol. 70, pp. 311-329.
- 1938 The fate of colloidal calcium phosphate in the dog. *Am. J. Physiol.* (In press.)
- McLEAN, F. C., AND M. A. HINRICHS 1938 The formation and behavior of colloidal calcium phosphate in the blood. *Am. J. Physiol.* (In press.)
- MUDD, S., M. McCUTCHEON AND B. LUCKÉ 1934 Phagocytosis. *Physiol. Rev.*, vol. 14, pp. 210-275.
- SCHMIDT, C. L. A., AND D. M. GREENBERG 1935 Occurrence, transport and regulation of calcium, magnesium and phosphorus in the animal organism. *Physiol. Rev.*, vol. 15, pp. 297-434.
- SHELLING, D. H. 1935 *The Parathyroids in Health and in Disease*. St. Louis. The C. V. Mosby Company.
- SHELLING, D. H., AND H. W. JOSEPHS 1934 Calcium and phosphorus studies. X. The effect of variation of calcium, phosphorus, and of vitamin D in diet on iron retention in rats. *Bull. Johns Hopkins Hosp.*, vol. 55, pp. 309-313.

PLATE 1

EXPLANATION OF FIGURES

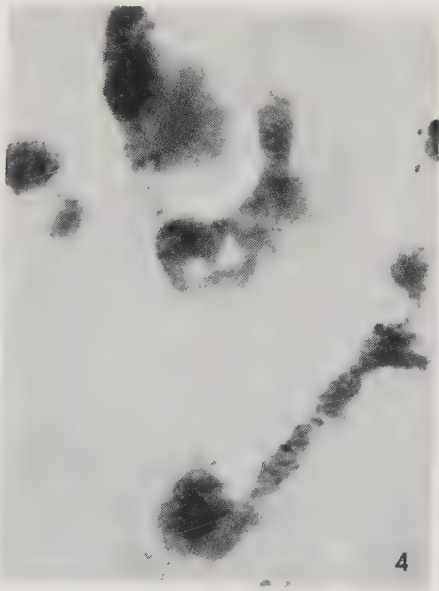
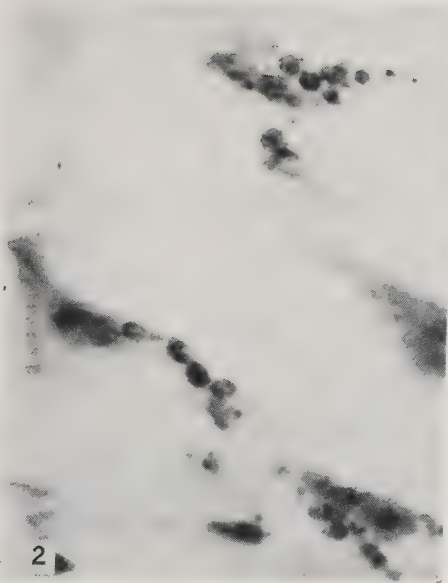
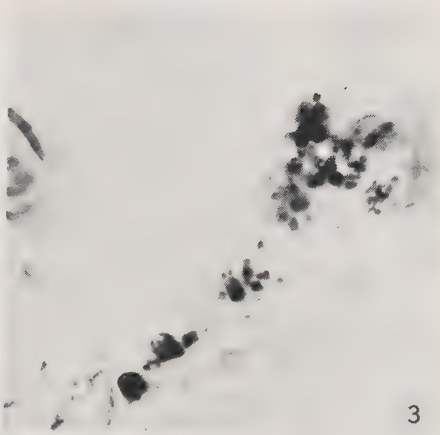
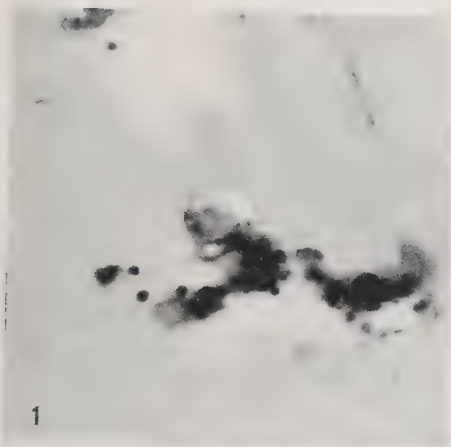
The black granules in all figures are colloidal calcium phosphate phagocytized by the Kupffer cells of the liver. The granules are found only in the cytoplasm of the Kupffer cells; none are in the parenchymatous liver cells. In the first three figures, the colloidal calcium phosphate was formed in the blood after the injection of soluble calcium or phosphate salts, or of both salts injected successively in the same animal; in the fourth figure, the colloidal particles were largely introduced as such into the blood. All preparations were treated with a silver reagent in such a way as to demonstrate phosphate and were then photographed at a magnification of about 2000 times, with an apochromatic oil immersion objective ($\times 90$) and a $10\times$ ocular.

1 Liver of a rat weighing 330 gm. injected intravenously with 4.62 mg. calcium as 2% calcium chloride solution, and sacrificed 15 minutes later.

2 Liver of a rat weighing 240 gm. injected intravenously with 9.6 mg. phosphorus as a 5% neutral solution of sodium phosphate, and sacrificed 15 minutes later.

3 Liver of a rat weighing 150 gm. injected intravenously with 4 mg. calcium as a 5% calcium chloride solution, followed directly by 3.8 mg. phosphorus as a 5% neutral solution of sodium phosphate and sacrificed 15 minutes later.

4 Liver of a rat weighing 170 gm. injected with 1.7 cc. of a colloidal suspension of calcium phosphate, and sacrificed 5 minutes later.



DIFFERENTIATION IN CULTURE OF PIECES OF THE EARLY CHICK BLASTODERM

I. THE DEFINITIVE PRIMITIVE STREAK AND HEAD-PROCESS STAGES

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ELEVEN FIGURES

INTRODUCTION

In the course of exploration of various isolation techniques, several fairly extensive series of short-time cultures of pieces of the chick blastoderm have been prepared and studied. The present communication deals with the definitive primitive streak and head-process stages.

There have been many recent experimental investigations of the organization of these stages in which isolation methods have been used. Wetzel ('24, '36) and Hoadley ('26) have used an in situ sectioning technique. Waddington and his collaborators ('32, etc.) have combined explantation methods with transplantation of embryonic parts. Waddington ('32, '35) has also at various times investigated the differentiation of large pieces of the blastoderm on plasma clots, as well as the morphogenesis of the whole blastoderm under these conditions; Waterman ('36) has briefly reported defect experiments using this method. Hoadley ('26 b), Willier and Rawles ('31), Hunt ('31, '32), Dalton ('35), Rawles ('36), to mention only a few contributions, have tested various parts of blastoderms in the axis-forming stage by means of the chorio-allantoic graft.

The preliminary question raised by the various results obtained in such isolations seems to me to be the following: In what degree do the structures differentiating from pieces

correspond to the normal axial layout of prospective areas as determined by vital staining, and how far are they to be ascribed to secondary reactions that may vaguely be designated as reconstitutions or inductions? Only when this question is answered is it possible to ask further ones concerning the independence or dependence of various organ fields, levels of the axis, germ layers, or other units, and their developmental role; or to evaluate the various experimental environments used.

The present experimental problem was to test *in vitro* areas of the blastoderm comparable to those whose behavior on the chorio-allantois is already known. Since, with the technique used, very small pieces of the blastoderm do not differentiate *in vitro*, it was decided to use only transverse strips of the pellucid area, the blastoderm being so divided into approximate fourths or fifths. In two of the series reported, the entoderm was left intact; in the other two, the upper layer (mesectoderm) was explanted separately. Pieces of this size are large enough to undergo morphogenetic changes when placed on plasma clots. As will be seen, axial structures arising under these conditions originate from their areas of prospective significance.

TECHNIQUE

White Leghorn and Rhode Island Red eggs were used; 16 to 22 hours' incubation gave the range of stages used. Eggs were opened in warm sterile Ringer, the blastoderm removed from the yolk and dissected in Pannett-Compton fluid kept at 38.5°, under a binocular microscope. Measurements of the length of streak, length of head process if present, or the distance from the pit to the anterior edge of the pellucid area, were made with an ocular micrometer. Transverse marks were made at the desired levels with a glass needle; the distance of these from the pit was then measured. If the entoderm were to be left intact, these marked out pieces were simply separated one from another, the opaque area trimmed off, and the piece transferred in a pipette to the surface of a cover slip clot made of two drops of plasma and two drops

of dilute embryonic extract. In the series where it was decided to remove the entoderm, the marked blastoderm was turned over in the dish. Almost invariably the light pressure of needle necessary to make marks on the ectodermal side had been sufficient to sever the delicate splanchnopleure completely. The mesentodermal strips could then be removed one by one, usually quite freely except at the streak or pit, where dissection would be necessary. These strips, incidentally, were also explanted; since their differentiation capacity was very slight and was limited almost entirely to mesodermal components also found in mesectodermal cultures, they will not be discussed here. The remaining heavy mesectodermal strips were trimmed and transferred to clots.

The Maximow double cover slip method was used. Cultures were run for 2 to 6 days; if for more than 3 days, they were washed in Pannett-Compton fluid and fed new plasma, or transferred to entirely new clots every other day.

OBSERVATIONS

1. *Capacities of various levels.* Figures 1 to 3 show the way the blastoderm was divided in the four experimental series. In the first series, diagrammed in figure 1,¹ the mesectoderm was explanted separately. The anterior cut was always made directly through the pit. The second cut was made one-fourth of the way back on the streak; this distance varied from 0.27 to 0.59 mm. posterior to the first, as indicated. The third cut was made halfway back on the streak, 0.59 to 1.00 mm. posterior to the pit.

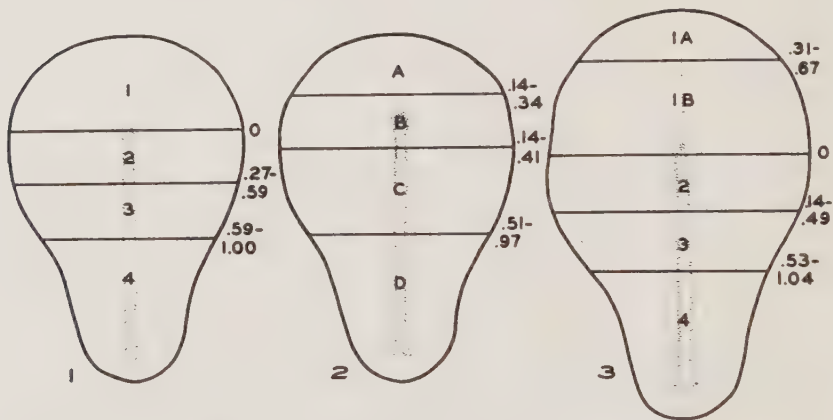
Figure 2 shows a variation used in the second and third series. In the second, the anterior cut was made 0.14 to 0.28 mm. anterior to the pit, the second cut 0.14 to 0.28 mm. posterior to the pit. The third was made 0.51 to 0.94 mm. behind the pit—again halfway back on the streak. The posteriormost piece was not explanted, being entirely like piece 4 in figure 1. The entoderm was removed in this series.

The third series was cut as in figure 2, the first cut 0.17 to 0.34 mm. anterior to the pit, the second 0.14 to 0.41 mm.

¹ This series was the subject of a preliminary report ('37).

posterior to the pit, the third 0.51 to 0.97 mm. back. The entoderm was included in this series, and all four levels—A, B, C, D—explanted. It will be noticed that in the foregoing two series the node was left intact, whereas in the first it was bisected transversely.

Figure 3 shows the cuts made on a small series of head-process blastoderms. The first cut was made approximately through the anterior tip of the head process. The second cut was through the pit; in a few, it was varied to a position



Figs. 1 and 2 Diagrams of cuts made on definitive primitive streak blastoderms; series 1 to 3; see text. Figures to the right represent distances from the primitive pit in millimeters.

Fig. 3 Diagram of cuts made on head-process blastoderms (series 4).

as far as 0.34 mm. posterior to the pit. The third was about one-fourth of the way back on the streak, the fourth halfway back. The entoderm was left intact in these cases, its removal being impractical.

Table 1 presents a summary of the structures found in the various types of piece explanted. It will be noted that the piece is designated as in figures 1 to 3, the series to which it belongs (*vide supra*) being indicated in the second column. The measured limits of the pieces can be seen by reference to the figures.

To take the levels in order, in the definitive primitive streak and very early head-process stage: anterior material, cut at least as far as 0.34 mm. anterior to the pit, may form medullary tube or plate, though not invariably. If the cut be made through the pit, the proportion of medullary differentiation

TABLE 1

Showing distribution of differentiated structures in definitive primitive streak and head process series diagrammed in figures 1 to 3 and described in the text. Figures under each heading show number of cases in which structure was identified; questionable cases are also indicated. 'Mesenchyme' is recorded only when formed inside a differentiated culture—not when migrated as fibroblasts. 'Epithelium' refers to columnar and cuboidal types; not to thin membranes. Endothelium is recorded only when clear-cut blood vessels are found

STAGE	SERIES	LEVEL	NUMBER OF CASES	MED. PLATE	SENSORY EPITHELIUM	BODY WALL	CHORDA	MESENCHYME	EPITHELIUM	HEART	ERYTHROBLASTS	ENDOTHELIUM
DPS	2	A	15	6	1; 1?	5		1				
	3	A	8	3		3		2	1			
	1	1	36	19; 2?	12; 1?	19	3	14	8	2?	1	1
	2	B	20	17; 2?	3	10	15; 3?	10	5	2; 1?	1	2
	3	B	10	8; 1?	4	8	9; 1?	10	1	2		5
	1	2	30	15; 2?	8	13	16; 1?	15	11	6	3	
	2	C	15	2; 1?		2		4	7	2?	7	3
	3	C	10	1; 2?		4		5	3	5; 2?	5	5
	1	3	26			10		21	3	2; 1?	14	4
	3	D	11								11	
	1	4	15					7	1		15	2
HP	4	1A	14	10; 1?	2	9	1; 1?	2	1			
	4	1B	16	11	1; 1?	13	13	8	3	9	7	
	4	2	7	1; 2?		1	1; 1?	4	2	3; 1?	2	
	4	3	13		1?	2; 1?		7	9		8	1
	4	4	6					2	4		6	3

increases, and a few dubious cases of heart muscle occur. In all this group, the presence of entoderm appears to have no effect on the differentiation of the upper layers.

The node level (B), if the node be left intact, produces medullary material nearly always. Functional heart muscle appears here rather strongly. Chorda is very frequent. An

effect of the entoderm appears at this level: it will be noticed in the second B group (series 3) that chorda is recorded in practically all cases: 95 to 100%, instead of 75 to 90% as in the mesectodermal group. Also, body mesenchyme occurs in 100% of the former group, and is better developed in almost every individual case. The removal of the entoderm in these pieces undoubtedly has a mechanically disruptive effect: it will be recalled that dissection was almost always necessary.

The post-nodal, anterior streak level (C) shows strikingly diminishing capacity for formation of medullary plate. Only the more inclusive C groups contain any of this tissue at all. These are all of inferior grade, some dedifferentiating, some very dubious. All are in pieces cut 0.21 mm. or less behind the pit. The 3 group, where the anterior cut was 0.27 mm. or more posterior to the pit, contains none at all. Chorda, as might be expected, is lacking in this group, as is sensory epithelium. The series containing entoderm (3) shows strikingly better heart differentiation; this of course is to be expected in view of the fact that the lower mesodermal layer usually adheres to the entoderm; the only surprising thing is that the difference did not appear previously, at the node level.

The posterior (D, 4) piece produces erythroblasts uniformly; nothing else besides a little mesenchyme and epithelium. These pieces frequently do not differentiate at all, as will be seen by comparing the number of cases in the 4 group with other series 1 groups. All these levels were explanted in equal numbers; the number of cases reported indicates the proportion of successful differentiating cultures obtained.

The head-process cultures show the same picture, in slightly expanded terms. The piece anterior to the process can form medullary tube, as do process and node levels (1B). These latter have strongly developed heart masses, frequently double. Chorda, body wall epithelium, and mesenchyme are regularly formed. The posterior half of the node (2) has rather poor capacity for forming medullary tissue and chorda; heart is more frequent. The posterior levels (3, 4) form mesenchyme, some epithelia and erythroblasts.

Thus a consistent picture is provided by the distribution of differentiated structures in these series. Medullary tube or plate is found regularly in node level strips, and for some distance anteriorly. This corresponds with the prospective significance of the anterior material; and the relations of the structures as they are observed differentiating confirm the interpretation that these medullary tubes actually correspond to the prospective brain region. Posterior to the node, however, the capacity for medullary plate formation falls off rapidly; most of the prospective medullary field at streak levels is incapable even of an attempt at forming medullary plate under these experimental conditions. The material classified as 'sensory epithelium' will be discussed subsequently; here it may be noted that this epithelium is formed with considerable frequency in the node and anterior levels in streak stages; relatively rarely in corresponding regions of the head-process blastoderm. The notochord arises only from the immediate vicinity of the primitive pit; heart from lateral material at and somewhat posterior to the node level. Body wall ectoderm is found in all but posterior pieces; mesenchyme and indeterminate epithelia all through the levels tested; blood-forming tissue may arise almost anywhere, although most strikingly in the posterior streak region.

2. *Differentiation of the explanted strips.* The form assumed by the explanted pieces, always established in its essentials after 24 hours in vitro, was absolutely characteristic for each level, although, of course, variations on the ground-pattern were abundant. Pieces anterior to the pit or process tip, whether of the 1, A, or 1A type, formed very large thin-walled vesicles, simple or complex. Usually such cultures contained, in addition, small dense vesicles found subsequently to be composed of medullary tube, i.e., forebrain (fig. 9).

Strips containing all or part of the node or head process behaved quite differently on the clot. The median region would thicken considerably, remaining opaque, very rarely forming a vesicular structure. In this mass, medullary tissue and notochord could sometimes be distinguished in the living

culture. The lateral ends of the strip would swell, becoming thin-walled vesicles which often contained pulsating masses of heart tissue. This sort of culture is illustrated in figure 4, where one lateral vesicle and a median one are shown; the other lateral end had failed to vesiculate. Figure 6 illustrates a section of such a culture.

Pieces of the 3 or C type underwent a rather striking morphogenesis, of the sort shown in figure 5. The median portion, instead of forming medullary plate, would become a dense cord. This, on later days, might become extremely long, thin and contorted. The lateral regions formed large thin-walled vesicles, sometimes with a little heart tissue inside, but usually quite empty except for a few delicate mesenchyme strands, cell nests, tubules or endothelial vessels (figs. 7, 8).

Posterior pieces (4, D) did not form vesicles in more than half the cases. These pieces remain homogeneous; the streak disappears; usually the explant flattens out as a dense membrane, with slight peripheral migration. When vesiculation does occur, the whole explant takes the form of a blister rather than of a complete spherical structure. The interior remains full of cells, which after a few days are seen to be erythrocytes. Posterior pieces, whether vesicles do or do not form, yield dense masses of erythroblasts, which acquire haemoglobin usually on the third or fourth day.

The changes in form described above are well marked by the end of the first day of cultivation. Variations in form usually may be traced to the failure of one lateral portion to vesiculate: in that case, migration sets in, and the region in question becomes a membrane closely applied to the surface of the clot (this is occurring in the culture shown in fig. 4). The presence of the entoderm appears to make no difference. The fate of these structures is somewhat diverse. Compact vesicles and masses of medullary tissue may persist through several washings or transfers, and histological differentiation take place, provided cell migration does not occur. Thin-walled vesicles usually collapse after a day or two, forming very thin membranes on the clot. Rarely, they may shrink, become

dense, and persist; differentiation of tissues has not been observed in these cases. Posterior pieces usually persist as dense membranes on the clot, with a minimum of migration.

As between strips from definitive primitive streak and head-process stages, the differences are about what might be ex-



4



5

Fig. 4 Photograph of a living culture of a mesectodermal strip of level 2 (fig. 1) after 24 hours explantation. Below is one lateral vesicle; above the median region, which is now beginning migration; the other lateral portion is cut off at the top; this was not vesiculated. $\times 40$.

Fig. 5 Photograph of living culture of a mesectodermal strip of level 3 (fig. 1) after 24 hours. The median axial region has formed the dark projection directed toward the lower right hand corner; attached are seen the large thin-walled lateral vesicles, containing dark mesenchyme and blood cores, besides a delicate membranous complex. $\times 40$.

pected. The medullary tubes developing from head-process explants are much more extensive than the others and are more apt to be of recognizable form, containing regions corresponding to optic vesicles, brain segments, etc. In general, only the

axis at and anterior to the node level undergoes a normal morphogenesis into axial structures under the conditions imposed; in head-process stages this region is of course of greater extent than in previous stages.

The features of primitive morphogenesis that remain unaltered by transverse section of the blastoderm may be summarized as follows: Median axial regions condense *in vitro* as they do in normal embryogenesis. Anterior and lateral regions (i.e., ventral and extra-embryonic) show marked vesiculation; thus the coelomic areas already have certain innate capacities. Node and pre-nodal regions contain the head and heart already localized.

As for the primitive streak, the following points seem significant: The second quarter of the streak shows its destined capacity for regression by its marked anteroposterior shortening *in vitro*. This level when left attached to the anterior part of the blastoderm, will show posterior growth of the streak as a little 'tail' (Waddington, '32; Wetzel, '36); when this region is left attached to the posterior part of the blasto-

Figures 6 to 11 are photomicrographs of sections through cultures. Fixation, Allen B-15; stain, Heidenhain's iron hematoxylin.

Fig. 6 Section through median and one lateral mass, series 1 culture, level 2, mesectoderm, grown 2 days. Right, central mass with thin medullary plate, mesenchyme, and some cords. Left, coelomic vesicle containing mass of incompletely differentiated heart muscle and some erythroblasts. $\times 75$.

Fig. 7 Section through one vesicle, DPS mesectoderm, level 3, series 1, cultured 2 days. Note double structure, thick mesenchymal wall. Inside, epithelial tubule, spaces and blood vessel. This vesicle contains the maximum structure produced by level 3. $\times 75$.

Fig. 8 Section through vesicle of 2-day culture, DPS mesectoderm, level 3, series 1. This is the usual picture; double vesicle of membranous epithelium; a little mesenchyme and blood inside. $\times 75$.

Fig. 9 DPS, series 1, mesectoderm, level 1; cultured 3 days. Vesicle of body wall ectoderm, containing lobed medullary tube. Note variations in thickness of medullary wall, and 'sensory epithelial' character of upper lobe; also strand of cells connecting medullary tube with outer layer. $\times 75$.

Fig. 10 DPS, mesectoderm of level 4, series 1, cultured 2 days. Blister of thin epithelium, containing fibroblasts and erythroblasts. $\times 75$.

Fig. 11 DPS, series 1, mesectoderm of level 2, cultured 2 days. Note curving medullary tube, partly underlaid by chorda (right); somite masses at lower left of chorda. Good development of mesenchyme and endothelial vessels. $\times 75$.

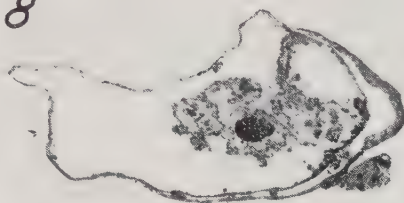
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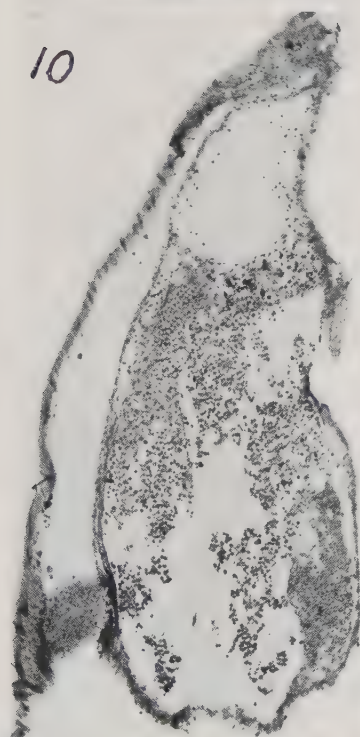
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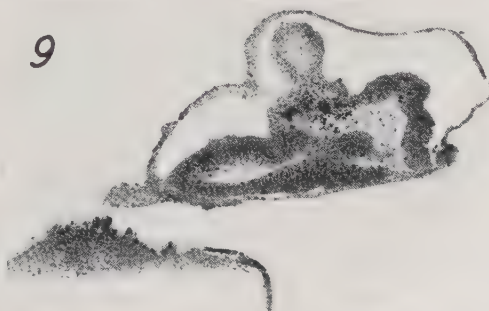
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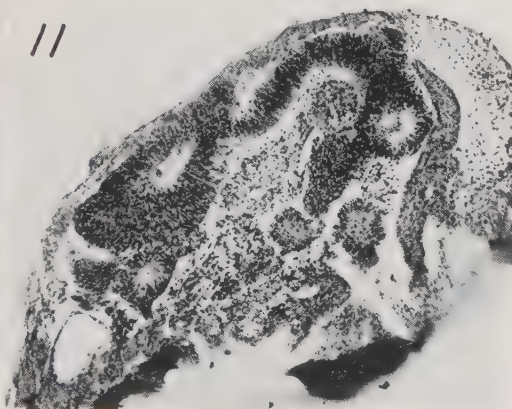
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11



derm, complete regression of the streak takes place (Waddington, '36). When the level is isolated, however, a sort of 90° deflection of growth takes place in the streak region. The condensed streak material becomes a cord connecting lateral vesicles; to do this it stretches tremendously in a lateral direction. This is accomplished, it seems from sections, much more by shape changes than by proliferation; it is not a direct result of tension in the cultures, since the cord may twist and even double on itself on the second or third day of explantation. The posterior half of the streak, evidently, possesses no such morphogenetic urge; it soon loses its identity in culture.

3. *Microscopic differentiation.* Figures 9 and 11 show the sort of medullary material developing in the cultures during the first 2 days of explantation. They are usually closed-off tubes, irregular in shape, with out-pocketings that may or may not be of morphological significance. In one case, such an evagination was found to be in contact with the outer ectoderm, and the contact was marked by a definite spherical thickening. This is undoubtedly an optic vesicle with lens; other similar formations are less sure of interpretation.

The medullary tubes or plates may vary in thickness, as comparison of the illustrations shows. There is a well-marked ependymal layer; on subsequent days of culture, ganglionic and fibrous zones may differentiate, although not in any regular pattern. This differentiation takes place only if the culture is transferred regularly, and at the same time prevented from migrating. Usually, a disorganization process sets in after 2 to 6 days; the medullary plates are broken up apparently by two processes: migration of cells from the plate, and over-proliferation within the plate itself—sometimes de-differentiating masses are found black with mitoses. In unhealthy cultures, or where the tissue mass is excessively bulky, necrosis may of course set in; but in the period under discussion the migration and proliferation factors are the major disrupting ones.

Frequently continuous with typical medullary plates (compare fig. 9) is a high pseudostratified epithelium very like that

normally forming the primordia of the sense organs—nose or ear. Some of this may actually be of such an extra-medullary origin; however, the relations of most of this epithelium, and the fact that it predominates in primitive streak stages and is relatively rare in the head-process series, makes one incline to interpret it as low-grade medullary plate in most cases.

Medullary and sensory epithelia, especially where tubes have formed, grow within vesicles of epithelium that resembles body wall ectoderm: low cuboidal type, with vacuolated cells. This differs from the thin membranes surrounding non-axial vesicles (compare figs. 9, 8): in section these latter are extremely thin, with almost no cytoplasm. More (fig. 11) or less (fig. 9) mesodermal structure may be interposed between the medullary tubes and the body wall. Figure 11 shows a rather extensive development of mesenchyme, notochord, somite masses, and blood spaces.

The notochord at first develops as a solid cord, which may or may not follow the course of the medullary tube, but which is almost always contorted. In some older cultures—after 3 days—the notochord may take on its characteristic vacuolated appearance; in others it never does this, but remains in its primitive condition.

Heart develops as little masses of muscle. In its earliest form, this tissue cannot be distinguished from mesenchyme; such masses may pulsate in vitro. Usually, a typical though minute mass of recognizable primitive myocardium is present. Interlacing myoblasts distinguish these masses; striations may develop after 3 days' culture. Figure 6 shows a typical general picture of a culture containing heart. To the left is the original median region of the strip, containing fragments of medullary plate. To the right is one lateral vesicle, containing the heart mass. Another heart vesicle, not shown in this section, is present on the opposite side.

Some sporadic formation of isolated erythrocytes seems to occur in certain anterior pieces; there are cases containing only a few cells of this type, usually in endothelial channels

or sacs. Posterior cultures tend to give a typical blood-island picture: dense masses of erythroblasts in endothelial vessels, although the latter may be absent. These masses are not always surrounded by an epithelial membrane, as in figure 10; but the position of the erythropoeitic masses suggests that their origin is mesodermal. The yellow color of haemoglobin is usually detected in the living explant during the third day of culture—a little later than its normal first appearance, but still while the erythroblasts are in their primitive form. Subsequently they may differentiate completely, and become dispersed in any space available.

The entodermal layer, in the series where it was retained, has shown no true differentiation. Occasionally a thin epithelial layer, or even a closed vesicle, is found, which has most probably been derived from the entoderm; but no structure beyond this.

DISCUSSION

The organization of the definitive primitive streak blastoderm takes on different aspects as different methods of analysis are applied. If the blastoderm be left on the yolk, and one or more cuts be made in it (Wetzel, '36), the isolated parts will, in general, develop according to their original intention; the movements of material in the streak region, and some other features of morphogenesis, are suppressed; but the main axial primordia develop in the position they held at the time the operation was performed. The same result is obtained if the whole blastoderm be explanted to a plasma clot (Waddington, '32) or if large pieces, as half or a third, be isolated on a clot (*ibid.*, '35; Waterman, '36): an embryo, or parts, arises from the material which would normally have formed these structures, even if the material is prevented from executing its usual movements. The posterior, or streak region of the axis is at a definite disadvantage when isolated from the node; nevertheless it may form primitive trunk parts independently. When the intact blastoderm is explanted, trunk and tail parts form very well. The movements of in-

vagination and regression of the streak, while not absolutely essential for the formation of the posterolateral medullary plate and somites, must account for the precision with which these events occur in the intact blastoderm. With the formation of the head process, there is no essential change in the behavior of the blastoderm when transected. Node and process levels form axis freely; streak levels do so with some difficulty when isolated, although streak movements are more uniform (Rudnick, '38).

When the blastoderm is tested by cutting it into pieces and grafting these to the chorio-allantois, a different picture is obtained. In the definitive streak stage, the head region—node level and anterior—consists of overlapping fields, of definite histogenetic potency, but more extensive and less definite in form than the prospective areas corresponding to the organs in question. The immediate vicinity of the node can form almost all axial tissues unaided. Behind the node, the prospective medullary field is weak in histogenetic potency (Hunt, '31, '32; Stein, '33; Dalton, '35; Rudnick, '38). In head-process stages, the fields previously so concentrated in the node vicinity become spread out to a position more nearly corresponding to their final site: lateral and posterior organs, in particular, become localized in this and later stages (Rudnick, '32, '35; Clarke, '36; Rawles, '36). The post-nodal prospective medullary area, however, does not acquire any further independence (Hunt, '31; Rawles, '36). Thus this analysis gives a picture of the blastoderm as a system localized only in the most general way in the node field and anteriorly, becoming progressively more specific during the head-process and subsequent stages (Willier and Rawles, '35).

The present experiments, like the other culture experiments and the *in situ* sections, show in the definitive primitive streak stage a localized axis in the anterior portion of the pellucid area, with the heart material, at least, already bilaterally situated. As in the chorio-allantoic analysis, capacity for medullary or nerve differentiation falls off very sharply behind the node. A transverse strip of the blastoderm at the level

of the second quarter of the streak, for example, will never form medullary plate *in vitro*, whereas the same material left attached to the whole posterior portion will form medullary plate in culture (Waddington, '35) and may even, infrequently (Dalton, '35; Rudnick, '38), form nerve tissue when grafted. Dalton finds that the presence of the entoderm is necessary for such differentiation; the present results suggest that the entirety of the posterior piece is also of importance, at least for the formation of medullary plate.

The present cultures also suffer restrictions in morphogenesis of mesodermal and entodermal derivatives; indeed, the dissection of the blastoderm appears to entail all the developmental limitations of all other methods. This is due apparently to mechanical block of movements in and of the streak.

In spite of a strong tendency to migrate and disorganize, the primitive structures formed in culture—medullary plate, heart, notochord, erythroblasts—are capable of completing their differentiation *in vitro* under suitable conditions. Burrows ('11) and Olivo ('27) have described the migration and differentiation of neurones from young medullary plate explants; Grigorieff ('31) describes the differentiation of nerve elements remaining in the original explant. The present cultures, if transferred, form fibrous areas distinct from ganglionic ones. No migration of neuroblasts has been observed: the cultures carried on to differentiation were selected because the outer layer remained intact.

Olivo ('28) has likewise observed, among other things, the differentiation of pulsating areas, and finally of striated heart muscle, from the prospective heart areas of the stages used here. Murray ('32) has described the differentiation of erythrocytes from all parts of the blastoderm except the anterior quarter of the streak. The histological differentiation of notochord in cultured total embryos has of course been described by Waddington ('32). Thus potencies for differentiation of cell types and small tissue units appear quite complete even in the early stages studied here.

SUMMARY

1. Short-time cultures of transverse fourths or fifths of definitive primitive streak and head-process blastoderms, either with or without the mesentodermal layer, were studied with reference to morphogenesis of early embryonic structures.

2. The node level and anterior region, in the stages studied, form medullary plate, body wall, notochord, and some mesoderm from their axial (median) portion, which undergoes relatively typical morphogenesis. Anterior and lateral non-axial regions form coelomic vesicles. Heart is localized laterally. Removal of the mesentoderm reduces the incidence of chorda and heart.

3. The second quarter of the streak forms no axial structures in vitro, although the streak tissue behaves in a striking and unique manner. Lateral regions at this level form vesicles. Presence of the mesentoderm makes no difference in the behavior of this region.

4. The level including the posterior half of the streak, whether entoderm is present or no, forms large masses of erythroblasts and a few non-specific cell types.

5. The structures formed may be histologically similar to those of a corresponding normal stage, or they may present deficiencies. The best cases, at least, are capable of complete histogenesis in vitro, although the tendency to dedifferentiation is strong.

6. Like the results obtained by the in situ sectioning method at the same stage, the present ones show a localized axis at the node and anterior levels; unlike the former, but like the results from chorio-allantoic grafts, they show complete lack of axial organization posterior to the node level.

LITERATURE CITED

- BURROWS, M. T. 1911 The growth of tissues of the chicken embryo outside of the animal body with special reference to the nervous system. *J. Exp. Zool.*, vol. 10, pp. 63-83.
- CLARKE, L. F. 1936 Regional differences in eye-forming capacity of the early chick blastoderm as studied in chorio-allantoic grafts. *Physiol. Zool.*, vol. 9, pp. 102-128.
- DALTON, A. J. 1935 The potencies of portions of young chick blastoderms as tested in chorio-allantoic grafts. *J. Exp. Zool.*, vol. 71, pp. 17-52.

- GRIGORIEFF, L. M. 1931 Differenzierung des Nervengewebes ausserhalb des Organismus. I Mitt. Arch. Exp. Zellf., Bd. 11, S. 483-519.
- HOADLEY, L. 1926 a The in situ development of sectioned chick blastoderms. Arch. de Biol., vol. 36, pp. 225-309.
- 1926 b Developmental potencies of parts of the early blastoderm of the chick. I-III. J. Exp. Zool., vol. 43, pp. 151-224.
- HUNT, T. E. 1931 An experimental study of the independent differentiation of the isolated Hensen's node and its relation to the formation of axial and non-axial parts in the chick embryo. J. Exp. Zool., vol. 59, pp. 395-427.
- 1932 Potencies of transverse levels of the chick blastoderm in the definitive-streak stage. Anat. Rec., vol. 55, pp. 41-70.
- MURRAY, P. D. F. 1932 The development in vitro of the blood of the early chick embryo. Proc. Roy. Soc. Lond., B, vol. 111, pp. 497-521.
- OLIVO, O. M. 1927 Migrazione di elementi nervosi coltivati in vitro. Arch. Exp. Zellf., Bd. 4, S. 43-63.
- 1928 Über die frühzeitige Determinierung der Herzanlage beim Hühner-embryo und deren histologische und physiologische Differenzierung in vitro. Anat. Anz., Bd. 66, Erg. Heft, S. 108-118.
- RAWLES, M. E. 1936 A study in the localization of organ-forming areas in the chick blastoderm of the head-process stage. J. Exp. Zool., vol. 72, pp. 271-315.
- RUDNICK, D. 1932 Thyroid-forming potencies of the early chick blastoderm. J. Exp. Zool., vol. 62, pp. 287-313.
- 1935 Regional restriction of potencies in the chick during embryogenesis. J. Exp. Zool., vol. 71, pp. 83-99.
- 1937 Differentiation in vitro of transverse strips of the mesectoderm of the chick blastoderm. Anat. Rec., vol. 67, sup. no. 3, p. 43.
- 1938 Contribution to the problem of neurogenic potency in post-nodal isolates from chick blastoderms. J. Exp. Zool. (In press.)
- STEIN, K. F. 1933 The location and differentiation of the presumptive ectoderm of the forebrain and hypophysis as shown by chorio-allantoic grafts. Physiol. Zool., vol. 6, pp. 205-235.
- WADDINGTON, C. H. 1932 Experiments on the development of chick and duck embryos cultivated in vitro. Phil. Trans. Roy. Soc. B, vol. 221, pp. 179-230.
- 1935 The development of isolated parts of the chick blastoderm. J. Exp. Zool., vol. 71, pp. 273-288.
- WATERMAN, A. J. 1936 Experiments on young chick embryos cultured in vitro. Proc. Nat. Acad. Sci., vol. 22, pp. 1-3.
- WETZEL, R. 1924 Über dem Primitivknoten des Hühnchens. Verh. phys. med. Ges. Würzburg, Bd. 49, S. 227-233.
- 1936 Primitivstreifen und Urkörper nach Störungsversuchen am 1-2 Tage bebrüteten Hühnchen. Arch. Entwmech., Bd. 134, S. 357-465.
- WILLIER, B. H., AND M. E. RAWLES 1931 The relation of Hensen's node to the differentiation capacity of whole chick blastoderms as studied in chorio-allantoic grafts. J. Exp. Zool., vol. 59, pp. 429-465.
- 1935 Organ-forming areas in the early chick blastoderm. Proc. Soc. Exp. Biol. and Med., vol. 32, pp. 1293-1296.

BOOK REVIEW

CHEMISTRY OF THE BRAIN. By Irvine H. Page, M.D.,
Hospital of The Rockefeller Institute for Medical Research, New
York, Charles C. Thomas, Springfield, Ill. and Baltimore, Md.
1937. xvii + 444 pp. \$7.50.

In the preface of this valuable source book on the chemistry of the brain, attention is called to the facility with which chemical differences between the input and output of the brain can be studied due to the accessibility of the arterial supply, venous drainage and cerebrospinal fluid. Five different approaches are suggested.

The successive chapters summarize the widely scattered literature in the following order: sterols, phosphatides, fatty acid metabolism, cerebroside, carbohydrates, nitrogenous metabolism, electrolytes and gases. Two chapters are devoted to metabolism of the central nervous system as measured by gas interchange under various influences such as mental work, narcosis, heat and cold, hypertension, epilepsy and other diseases.

The important subjects of water balance and water intoxication are considered in a chapter on physical chemistry which also takes up the distribution of various narcotics, hypnotics and other drugs within the brain. The value of studying the molecular anatomy of nervous tissues by the x-ray diffraction method is pointed out. A short chapter on enzymes of the brain concludes with a tabulation of their action and associated pathological processes.

To morphologists the chapter on the comparative and developmental neurochemistry is of special interest. It gives considerable qualitative and quantitative data on the distribution of various chemical constituents in the nervous system of man and various animals during different stages of development and at various ages. Diet, vitamins and degeneration of the nervous system with associated chemical changes are other topics of value to experimental morphologists. The probable intermediate and final products in the demyelination process are briefly considered.

The fifteenth chapter, written by J. H. Quastel of the Biochemical Laboratory, Cardiff City Mental Hospital, Wales, is on oxidation-reduction. It summarizes the current views on the mechanism of oxidation in living cells, the behavior of dehydrogenase and the effect of various chemicals and ionic environments on brain respiration.

The final chapter, on 'The Brain and Thought,' while necessarily philosophical, is justified by the assumption that thought may be considered as a manifestation of energy, the liberation and coordination of which is a function of the brain, and that the kinetics of the brain can be altered by variations in the chemical processes going on within its substance.

Each chapter is headed by a general bibliography and specific references are given in extensive footnotes. Clinical applications are brought in throughout the book, which concludes with a well-arranged index. The type, binding and paper are all excellent.

On page 237, in connection with the distribution of iodine in the central nervous system (quoting Schittenhelm, Eisler, Sturm and Schneeberg) the common mistake is made of translating *Zwischenhirn* as midbrain instead of twixt-brain (diencephalon).

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NEW BOOKS AND PUBLICATIONS

ILLUSTRATIONS OF REGIONAL ANATOMY, by E. B. Jamieson, M.D., senior demonstrator and lecturer, anatomy department, University, Edinburgh. 1937. Seven sections, five of which have been revised for the second edition. 305 plates, most of them in colors, $6 \times 4\frac{1}{4}$ inches. The plates for each section are bound in a loose-leaf binder. Sections may be purchased separately. Price: Section I, Central Nervous System, 48 plates, \$2.50; Section II, Head and Neck, 63 plates, \$3.50; Section III, Abdomen, 37 plates, \$2.25; Section IV, Pelvis, 33 plates, \$1.50; Section V, Thorax, 30 plates, \$1.75; Section VI, Upper Limb, 42 plates, \$2.50; Section VII, Lower Limb, 52 plates, \$3.50; Complete set (seven sections) 305 plates, \$15.00. William Wood and Company, Baltimore.

The revised and improved edition of the first five sections, together with the sixth and seventh sections which were issued as a sequel to the original edition, comprises an up-to-date atlas which has no superior for the study of human anatomy.

THE GOLGI APPARATUS IN THE PROXIMAL AND DISTAL TUBULE CELLS OF THE PERFUSED FROG'S KIDNEY

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TEN TEXT FIGURES AND ONE PLATE (EIGHT FIGURES)

INTRODUCTION

Recent observations made on cells grown in tissue cultures have shown that in many instances the Golgi apparatus of these cells is abnormal. Investigations by Ludford ('27), Richardson ('34), Macdougald and Gatenby ('35), and Macdougald ('35) on a variety of tissues have demonstrated that the Golgi apparatus undergoes a series of transformations including its fragmentation and apparent metamorphosis into globules of fat. More recently Macdougald ('37) has reviewed the subject and from further studies has reported that under proper conditions a normal form of the Golgi apparatus persists throughout extended periods of growth in vitro, although it may at any time undergo degeneration and fragmentation. Through the application of fat staining to his preparations he comes to the conclusion that the fat in degenerating cells does not arise from or in relationship with the fragmenting Golgi apparatus.

The purpose of the present study was to determine what changes take place in the Golgi apparatus of the tubule cells of the frog's kidney when whole animals are perfused with oxygenated Ringer's solution. The observed changes are in many respects comparable to those seen in tissue culture, the chief difference being that a decrease in the amount of Golgi material was not accompanied by a corresponding appearance of fatty droplets.

TECHNIQUE

Male specimens of *Rana pipiens* were used in these experiments. They were obtained from a dealer, and following their arrival were kept without food for a period varying from a few days to several weeks in a moist, dark tank.

Whole animals were perfused through the aorta with a pulsating flow of oxygenated Ringer's solution which for each experiment was freshly prepared from chemically pure reagents and glass redistilled water. The brain and anterior portion of the spinal cord of an experimental animal were destroyed by pithing, the heart exposed, and the aorta cannulated by way of the ventricle. The flow of the perfusate was then established and the auricles cut to allow its escape. The

TABLE 1

TIME OF PERFUSION		TOTAL PERFUSATE	pH	WEIGHT OF ANIMAL	EXPERIMENT NO.
<i>hours</i>	<i>minutes</i>	<i>cc.</i>		<i>gm.</i>	
	30	350	7.38	49	137
1	30	300	7.38	43	138
3	45	1000	7.35	55	139
5	45	2750	7.5	..	131
8	40	4500	7.35	42	140-B

mean pressure was adjusted to about 40 mm. of mercury, with a pulsatile pressure of about 10 mm. of mercury and a pulse rate of 45 beats per minute. Bieter and Scott ('28) report systolic pressures of from 25 to 35 and diastolic pressures of from 18 to 28 mm. of mercury as normal for frogs. The somewhat higher pressures used in the present experiments seemed desirable in order to increase the amount of oxygenated fluid passing through the animal.

Immediately after each perfusion small sections of kidney were removed from corresponding regions and osmicated according to Nassanov's modification of Kolatchew's technique. The tissues were dehydrated by means of three 2-hour changes of dioxan and embedded in paraffin. Material treated in this way was in every respect as satisfactory as that dehydrated

through a series of alcohols and yielded good impregnations requiring no bleaching. Table 1 contains data pertaining to the individual perfusions.

OBSERVATIONS

General condition of animals during perfusion. Since the perfusion fluid contained no colloidal constituents to supply an osmotic pressure comparable to that furnished by the plasma proteins, the experimental animals developed varying degrees of edema during the course of perfusion. Although usually confined to the subcutaneous lymph sacs, in some of the longer perfusions the edema frequently involved the viscera and tongue. The kidneys of even the most edematous animals, however, appeared to have normal dimensions, and the sections later showed no evidence that the close packing of the tubules had been altered.

At the end of all perfusions a vigorous leg jerk could be obtained by mechanically stimulating the sciatic nerve; and in some of the animals in which only the most anterior portion of the cord was destroyed, spinal reflexes persisted for as long as 3 hours during perfusion. A cell in metaphase was observed in kidney tissue of an animal perfused for more than 5 hours.

Darlington ('37) has found that a 10-minute perfusion with trypan blue in a concentration of 1:4000 in Ringer's solution affords a means of distinguishing normal from dead or dying cells. The dead or severely injured cells stain a diffuse blue, while the uninjured cells remain unstained. In a number of preliminary experiments this technique was employed following perfusions of about 45 minutes duration. Although many of the proximal convoluted tubules showed considerable distension and some erosion of the brush border, neither these cells nor cells of the distal convoluted tubules were stained by the dye. On the basis of Darlington's results it can be assumed that exposure to the perfusion fluid for at least this length of time has not brought about a sufficiently marked alteration from the normal physiological condition of the kidney to cause severe injury or death of the cells.

These considerations are of importance in relation to the question of the condition of the tissues after prolonged perfusion. It is not possible to define exactly the state of the kidney cells examined in this study, for there are no accurate criteria of normality. A comparison was made, however, between the changes occurring in the Golgi apparatus in the renal cells of the perfused animals and those occurring in an excised kidney kept in a moist chamber. This experiment will be further discussed below, but for the present it may be pointed out that the differences in the reaction of the Golgi apparatus in the excised and perfused kidneys indicate that those occurring in the latter cannot entirely be explained on the basis of cell death.

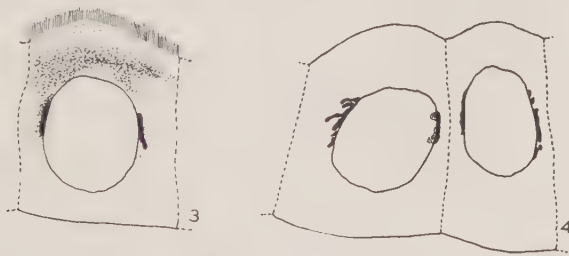


Figures 1 and 2

Golgi apparatus in the normal kidney. Chief attention in the present observations is directed to the brush border cells of the proximal convoluted tubules and the cells of the distal convoluted tubules containing the so-called batonnets of Heidenhain. In order to establish a basis for evaluating the changes observed in the perfused tissues it is necessary to describe briefly the form and variability of the Golgi apparatus in these two regions of the normal frog's kidney.

The variety of appearances presented by the Golgi apparatus in the brush border cells has been observed and described in the frog by Pappenheimer ('16) and in other Amphibia by Feyel ('28). In all but very rare instances it is definitely polarized distal to the equator of the nucleus. In

some cells the Golgi material is in the form of a few strands or irregularly shaped masses usually oriented parallel to the apico-basal axis of the cell, and in some instances apparently lying free in the cytoplasm distal to the nucleus (fig. 11). More typically, however, the Golgi apparatus appears to consist of nodulated strands or compact masses lying close to the nucleus. These larger masses usually are not homogeneously impregnated, for they frequently appear to be composed of darker and lighter components. The more darkly impregnating material is usually in the form of smaller masses united by delicate, anastomosing filaments with the more lightly impregnating material occupying the interstices. Filamentous strands of Golgi material often extend out into the



Figures 3 and 4

cytoplasm (figs. 1, 12). That the Golgi material frequently surrounds the distal half of the nucleus can clearly be seen in a cell sectioned at right angles to its apico-basal axis.

The Golgi apparatus in cells of the distal convoluted tubule is shown in figures 2 and 13. Here again its form is quite variable, but the osmiophilic structures usually appear as reticula or several compact masses in the supranuclear region. Their position in these cells is markedly more apical than in the cells of the brush border segment.

Perfusion for 30 minutes. A diffuse darkening of cells of the brush border segments is in marked contrast to the appearance of the batonnet segments. In some tubules this darkening is general throughout the cells, while in others it is most conspicuous in the region just distal to the nucleus (figs. 3, 14). The cells retain their cuboidal form, and the brush

border in most instances is intact. The subcuticular region is frequently filled with vacuoles of various sizes. The Golgi material in the brush border cells appears considerably reduced in amount; and although its position is variable, it frequently shows a marked tendency to be closely applied to and flattened against the nuclear membrane.

In some cells of the batonnet segments the Golgi apparatus appears as in the normal kidney. In many cells, however, it shows a shift from its typically apical position and a tendency to lie lateral to the nucleus, frequently in contact with the nuclear membrane (figs. 4, 15).

Perfusion for 1 hour and 30 minutes. The brush border is intact in all but a few instances. The darkening of these cells is more pronounced than after the 30-minute perfusion, and

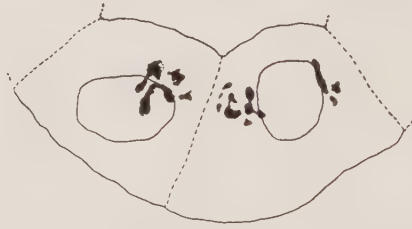


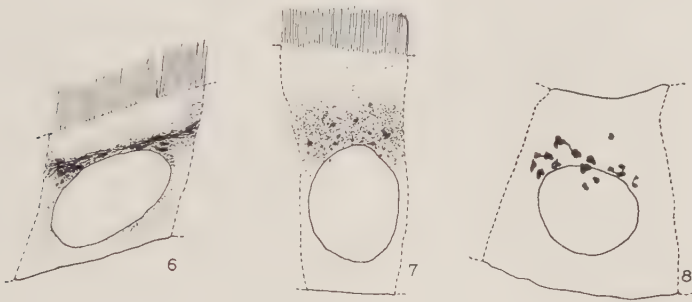
Figure 5

many show coarsely granular cytoplasm. In some cells the darkening is largely confined to a narrow band in the subcuticular zone, which in cross section of a tubule appears as a concentric ring. A Golgi apparatus could be identified in only a few of the less darkened cells, and in these it appears as a few strands or fragments which are located at random distal to the nucleus and are not sharply delimited. These conditions can be seen in figures 6 and 7, which are taken from an animal perfused for 3 hours and 45 minutes.

In some cells of the batonnet segment the Golgi apparatus is compact and in close relationship with the nuclear membrane, as frequently observed in the 30-minute perfusion. The typical appearance, however, is that of a loose, nodulated, or coarsely granular structure free in the cytoplasm lateral to the nucleus (fig. 5).

Perfusion for 3 hours and 45 minutes. The brush border of many of the proximal tubules shows considerable erosion. The Golgi apparatus, if present in these cells, is so modified that its identification is very uncertain. In figure 7 is shown a cell with diffuse darkening in the supranuclear zone, where a few more darkly impregnating granules probably are fragments of Golgi material. Figure 16 shows the marked contrast between the appearance of the distal tubules and the darkly impregnating proximal tubules.

The Golgi apparatus in the batonnet cells shows an increased amount of fragmentation. Some cells show little change from the conditions previously observed, but in a



Figures 6, 7 and 8

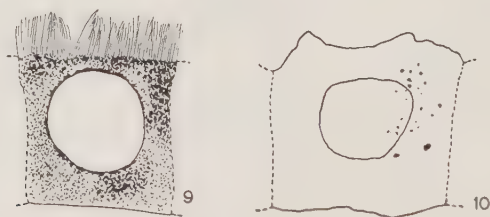
larger number the fragments of Golgi material are smaller and tend to lie more distal in the cell (fig. 8).

Perfusion for 5 hours and 35 minutes. The cytoplasm of the brush border cells is darkly impregnated. The epithelium of many tubules is reduced to one-third or one-fourth of its normal height; also the diameter of the tubules has increased slightly, and the nuclei have become elongated in a direction concentric with the lumen. These appearances suggest that the tubules are being distended (fig. 17). The brush border of many tubules is intact, while others show severe erosion. Some tubules in cross section appear to be completely filled with plugs of loose cells arranged in more or less concentric layers. It is possible that these cells dam the tubules, thus

giving rise to the observed distension. Such cell masses are occasionally seen in a normal kidney, where they possibly represent a stage in the development of new tubules, although the latter seems unlikely in the perfused animals.

The batonnet cells show a marked decrease in the amount of Golgi material. A few fragments varying in size usually persist either free in the cytoplasm or in contact with the nuclear membrane (fig. 17). Many of these cells, which otherwise appear unaltered, show no evidence of a Golgi apparatus.

Perfusion for 8 hours and 40 minutes. The brush border segments are dark and coarsely granular, and with the exception of scattered fragments in a few cells show no typical Golgi material (fig. 9). The presence of sloughed off cells



Figures 9 and 10

within the tubules and in the ureter provide further evidence of disintegration.

Some of the batonnet cells still contain a few fragments of the Golgi apparatus (fig. 10). They are found either in the cytoplasm in the region of the nucleus or closely applied to the nuclear membrane.

Resumé of observations. From the preceding description it is evident that the Golgi apparatus in the renal tubule cells during perfusion undergoes a series of modifications and eventually disappears. The observations can be summarized as follows:

1. In cells of the proximal convoluted tubules the Golgi apparatus normally appears as strands or reticular masses of osmiophilic material free in the apical region or in contact with the nuclear membrane. From the juxtanuclear portions

strand-like extensions frequently protrude into the cytoplasm. Soon after perfusion begins these extensions of the Golgi material disappear, and the remainder is found closely applied to the lateral surface of the nuclear membrane. The Golgi material continues to diminish in quantity until only a few granular fragments remain distributed in the supranuclear region, and these ultimately disintegrate and disappear. Concomitant with the disintegration of the Golgi apparatus, the cell as a whole becomes altered in such a way that it is diffusely darkened when treated with osmic acid. This darkening, which first appears in the subcuticular region, soon involves the whole cell, although it usually remains more intense in the apical region.

2. Somewhat comparable changes take place in cells of the batonnet segment. The Golgi apparatus moves from its typically apical position and becomes closely applied to the lateral surface of the nuclear membrane. This is followed by a period of fragmentation during which some of the fragments remain in contact with the nuclear membrane, while most of them move out into the cytoplasm lateral and distal to the nucleus. The fragments then decrease in size and number and ultimately disappear.

Two outstanding differences between the disintegrative processes in the proximal and the distal tubules are apparent. In the proximal tubules disintegration occurs more rapidly than in the distal tubules. Furthermore, fragmentation and disappearance of the Golgi apparatus in the latter is not accompanied by the diffuse darkening which is characteristic of these changes in the former. The possibility presents itself that these differences in reaction may be associated with the different physiological activities in these two regions of the tubule.

Autolysis at room temperature. Inasmuch as the possibility exists that some of the changes during perfusion may be due simply to autolytic processes, it was desirable to compare these changes with those occurring during autolysis not involving perfusion. While no exhaustive study of autolysis

was undertaken, observations were made of the changes taking place in the Golgi apparatus in an excised kidney. The organ was placed in a covered Petri dish containing a wad of cotton moistened with Ringer's solution. The latter was merely to prevent drying and was not in contact with the kidney. Pieces of tissue were removed and fixed at intervals up to 23 hours. Under these conditions the course of events in the later stages of autolysis may have been influenced by the growth of bacteria.

At the end of 1 hour cells of both proximal and distal tubules are in various stages of disintegration. The brush borders of many are severely eroded and stringy in appearance, while the cytoplasm is coarsely granular and contains many clear areas. In the distal tubules the cytoplasm likewise has undergone marked degeneration. Most of the cells have lost their striated appearance and now contain coarse and fine granules distributed irregularly. Many nuclei are shrunken and surrounded by a clear region. These changes become more pronounced and more general throughout the tissue in the pieces which were fixed after longer periods of time.

The changes in the Golgi apparatus differ in some respects from those observed in the perfused tissues. In the autolyzed brush border cells the Golgi apparatus, for some time during the initial stages of cytoplasmic disintegration, retains its normal position and form, as observed in the tissue fixed 1 hour after removal. Soon thereafter, however, it begins to break up into coarse fragments which become rather uniformly distributed in the apical region where they appear very distinct and sharply outlined against the cytoplasmic background (fig. 18). The fragmentation occurring here is in marked contrast to that which occurs during perfusion, for in perfused kidneys which showed various stages in the disintegration of the Golgi apparatus no cells contained the large number of coarse fragments which was typical of the cells undergoing autolysis. From a study of the perfused tissues it would seem that when fragmentation begins it proceeds rapidly, and that the Golgi apparatus quickly becomes reduced

to dust-like granules distinguishable with difficulty in the darkly impregnating supranuclear zone.

The form of the Golgi apparatus in the autolyzing batonnet cells was so varied that it was impossible to determine whether or not there is a sequence of changes which it undergoes during disintegration. In many cells the Golgi material is in the form of very irregular, attenuated strands or webs; in others it appears as a few granular masses which often are united by delicate anastomosing filaments. What appears to be the last phase in the disintegration of the Golgi apparatus is the loss of a distinct boundary by the few granules remaining in the supranuclear region and their dispersal as a dust-like material in a somewhat localized region immediately beneath the cell wall. From the extreme variability of the Golgi apparatus in the autolyzing cells it would appear that there probably does not occur a definite series of changes typical of its disintegration in all the cells, but that there is a fundamental process of fragmentation which is subject to much modification depending upon a number of factors the nature and action of which are at present not clear.

DISCUSSION

During perfusion, particularly with as simple a fluid as Ringer's solution, the tissues are being subjected to a highly abnormal environment. The cellular responses to the new environmental conditions are undoubtedly complex in nature, but insofar as the perfusion fluid is concerned in these changes its principle action is probably in the physico-chemical process of erosion by solution and washing out of various organic and inorganic constituents. Along with these effects there occur the autolytic processes taking place within the tissues as a result of cell injury and death under the influence of the perfusion fluid. Perfusion with a synthetic fluid such as Ringer's also deprives the tissues of their normal supply of nutrient materials, and it is possible that some of the observed changes

may be associated with a catabolic utilization of stored substances within the cells. The disintegration of the Golgi apparatus of cells grown in tissue culture has been attributed by recent investigators to the accumulation of waste products in the culture medium (Richardson, '34; Macdougald, '35). It would seem that perfusion with a continual supply of fresh solution, as in the present experiments, would remove such products as may be formed and reduce to a minimum their action as a factor influencing the disintegration of the Golgi apparatus.

On the basis of the present data it was not possible to decide which influences predominate in bringing about the changes observed to take place in the Golgi apparatus of the perfused cells. It is apparent, however, that the total effect of the perfusion is to bring about a definite series of alterations which includes fragmentation, dispersal, and eventual disappearance of the Golgi material; and that these changes differ in some respects from those occurring when only autolytic processes are involved. In view of the fundamental similarity of the two groups of observations it would seem that the ultimate disappearance of the Golgi apparatus in the perfused cells is a result of autolysis, but that the changes observed during the early period of perfusion arise from an interaction between the cells and the circulating solution.

The disintegration of the Golgi apparatus in the perfused cells is in some respects similar to that observed in cells cultured *in vitro*. Macdougald ('35) states that in chick cardiac muscle the Golgi apparatus "hypertrophies and flattens, becomes applied to the nuclear membrane, appears to 'flow' around the nucleus during which fragmentation ensues, the fragments becoming fairly regularly distributed around the nucleus, and finally they metamorphose into fat droplets which coalesce to form larger globules." These observations and those of Richardson ('34) both emphasize the close association between Golgi apparatus and the nuclear membrane. From the material examined in the present study there is considerable evidence that during perfusion with

Ringer's solution one of the early changes in the Golgi apparatus is its movement into close approximation with the nuclear membrane. During the ensuing fragmentation this close association does not persist, and most of the fragments move out into the cytoplasm distal or lateral to the nucleus.

Metamorphosis of the Golgi material into fat was not evident in the perfused specimens. This certainly did not occur in the distal tubules, since many cells after more than 8 hours perfusion showed little obvious change other than that the Golgi material was reduced to a few small fragments or was entirely lacking. In the proximal tubules, on the other hand, there may be some evidence of lipoid formation. It might be that the diffuse darkening of these cells is due to the general liberation of a lipoid substance throughout the cell as a whole, although the more intense reaction in the apical zone would seem to indicate that in this region fragmentation of the Golgi apparatus is a contributing factor. In no instance, however, was there a coalescence to form large fatty globules as occurs in degenerating cells *in vitro*.

Macdougald's ('37) recent study has led him to the conclusion that the fatty droplets which accumulate in degenerating cells in tissue cultures arise independently, and not as previously believed, by transformation of fragments of the disintegrating Golgi apparatus. Insofar as the present observations bear on this question they present further evidence that the fragmenting Golgi apparatus does not metamorphose into fat.

SUMMARY

1. Frogs were perfused for various lengths of time with a pulsating flow of oxygenated Ringer's solution.

2. In the proximal and distal tubules of the kidney the Golgi apparatus undergoes a series of changes. In both regions the Golgi material becomes closely applied to the lateral surface of the nuclear membrane. This is followed by a period of fragmentation during which most of the fragments come to

lie distal or lateral to the nucleus, where they decrease in size and ultimately disappear.

3. Disintegration of the Golgi apparatus occurs more rapidly in the proximal than in the distal tubules, and in the former is accompanied by the appearance within the cells of a substance which is diffusely darkened by osmic acid.

4. Metamorphosis of the Golgi material into fatty droplets was not observed.

5. The changes during perfusion differ in some respects from those occurring during autolysis in an excised kidney.

I take this opportunity to express my sincere thanks for the helpful advice and scholarly criticism of Prof. J. Walter Wilson, who suggested this work.

LITERATURE CITED

- BIETER, R. N., AND F. H. SCOTT 1928 Blood pressure and blood protein determinations in the frog. *Proc. Soc. Exp. Biol. and Med.*, vol. 25, p. 832.
- DARLINGTON, J. M. 1937 The use of trypan blue in detecting cell death in the perfused mammalian kidney, and the evaluation of some modified Ringer-Locke fluids by this method. *Anat. Rec.*, vol. 67, p. 253.
- FEYEL, P. 1928 Des constituants de la cellule rénale chez quelques vertébrés. *Arch. d'anat. micr.*, T. 24, p. 359.
- LUDFORD, R. J. 1927 The Golgi apparatus in the cells of tissue cultures. *Proc. Roy. Soc., London*, vol. 101-B, p. 409.
- MACDOUGALD, T. J. 1935 Further studies on the abnormalities of the cytology of the growth zone in tissue cultures. *Arch. f. exp. Zellforsch.*, Bd. 18, S. 85.
- 1937 The Golgi apparatus of cells in tissue cultures. *Arch. f. exp. Zellforsch.*, Bd. 20, S. 35.
- MACDOUGALD, T. J., AND J. B. GATENBY 1935 'Growth zone' cytology in tissue cultures. *Arch. f. exp. Zellforsch.*, Bd. 17, S. 325.
- PAPPENHEIMER, A. M. 1916 The Golgi apparatus. *Anat. Rec.*, vol. 11, p. 107.
- RICHARDSON, K. C. 1934 The Golgi apparatus and other cytoplasmic structures in normal and degenerate cells in vitro. *Arch. f. exp. Zellforsch.*, Bd. 16, S. 100.

PLATE

PLATE 1

EXPLANATION OF FIGURES

Photomicrographs: magnification $\times 1000$.

11, 12 Normal proximal tubules showing variability of form and position of the Golgi apparatus.

13 Normal distal tubule. The Golgi apparatus is typically more apical than in the proximal tubule.

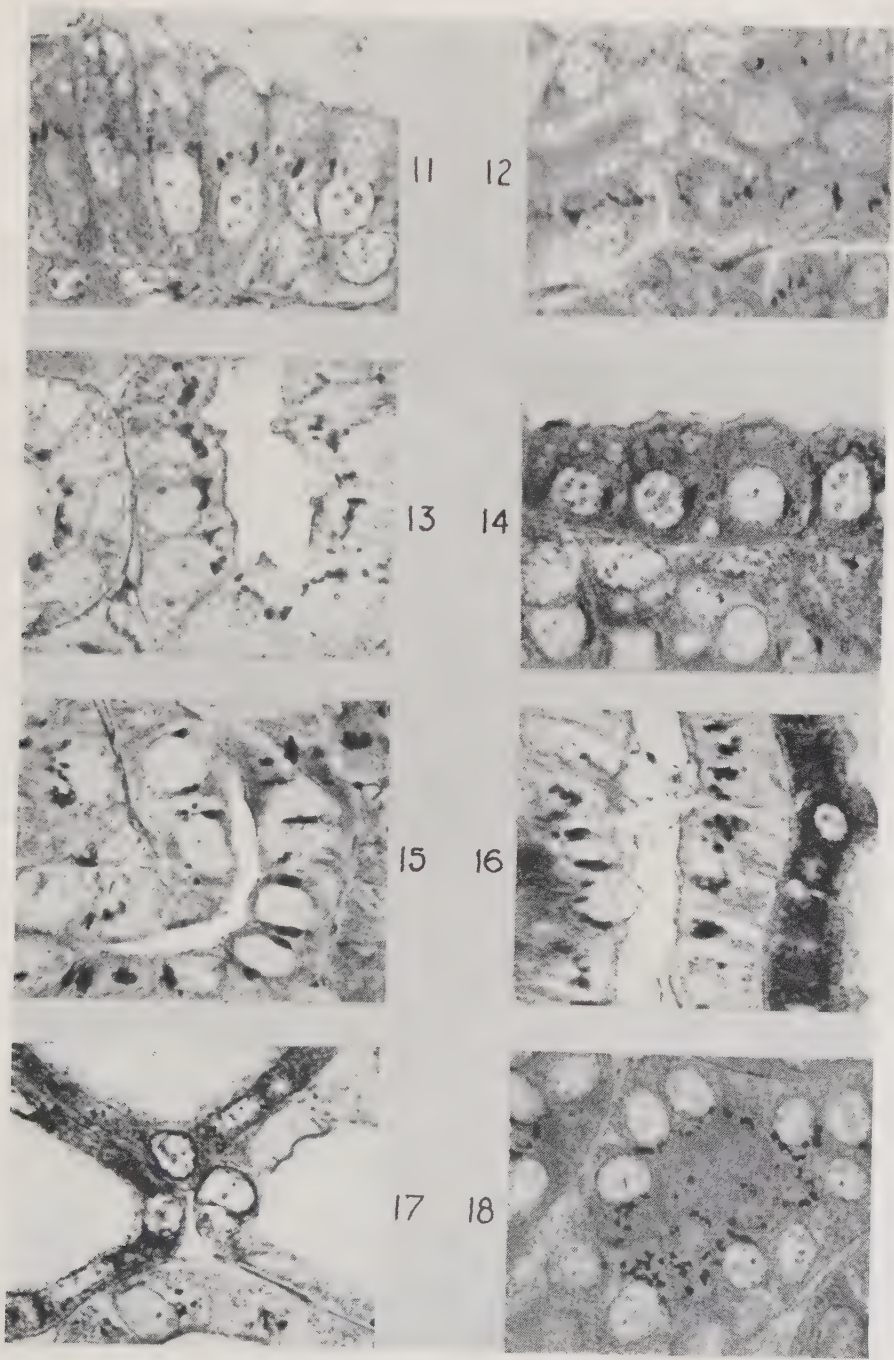
14 Perfused 30 minutes. Proximal tubule showing cytoplasm diffusely darkened and Golgi material applied to lateral surface of nuclear membrane.

15 Perfused 30 minutes. The Golgi apparatus has moved from its apical position and become applied to the lateral surface of the nuclear membrane.

16 Perfused 3 hours and 45 minutes. Note the contrast between the distal tubule and the intensely darkened proximal tubule. The Golgi apparatus in the former has begun to fragment.

17 Perfused 5 hours and 35 minutes. Proximal tubules showing distension as evidenced by lowered epithelium and elongated nuclei. A few fragments of Golgi material can be seen in cells of the lower distal tubule.

18 Autolyzed 12 hours. Proximal tubule showing coarse fragments of Golgi material located at random in the supranuclear region.



AGE ORDER OF EPIPHYSEAL UNION IN THE GUINEA PIG

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ONE FIGURE

The study of age changes in animal skeletons has become valuable experimentally since it offers a new comparative scale by which growth and development may be followed. First, the appearance of ossification centers, next, changes in the contour of epiphyseal outline, and finally, epiphyseal union cover a range of progress from infancy to maturity.

The work presented in this paper deals with the pattern of epiphyseal union in the guinea pig and seeks to establish a scale by means of which development may be measured in experiments which affect growth or maturation of the animal.

The guinea pig, rather than the rat or mouse, was chosen because of the greater ease in handling. In living rats and mice readable x-rays are difficult to obtain without special radiographic equipment. The second factor in this choice lies in the relatively long growth period in the guinea pig, thus affording opportunity for a large series of significant stages for the elaboration of a standard pattern of epiphyseal union.

Henle's observations on adolescent epiphyseal union (1871) as seen in the dead were the most reliable until the complete revision by Stevenson was published in 1924. This revision, made at Doctor Todd's suggestion, was carried out in this laboratory on our material. It showed that epiphyseal union occurs in a definite order, and that there is considerable uniformity in the age at which each epiphysis unites. Stevenson also notes that union of epiphyses of limb bones is earlier and

more regular than that of the vertebrae. The exhaustive studies made by Todd ('30 a, b, '31, '37) not only set down an accurate record in man but point to a means of estimation of the stage in maturity shown by young individuals before union commences. Dawson, in a series of papers published between 1925 and 1934, demonstrates patterns of union in albino rats of The Wistar Institute stock ('25, '27, '34 a). From his observations there is a marked similarity in order of union to that of man as reported by Stevenson. Dawson also confirmed the difference in regularity between closure in long bones and that of the axial skeleton. Furthermore, in his work there were two regions where closure sometimes did not occur even in 5-year-old animals. These were the heads of the humeri and femora.

To settle the point Dawson, in 1929, at Todd's suggestion, studied these regions histologically and concluded that he was dealing with 'lapsed union,' a disturbance described by Todd ('33) in a man with an osteoma of the pituitary fossa, who, at the age of 39 years, had epiphyses corresponding to the human 19-year level. These epiphyses showed pathological changes. Though growth had long ceased the epiphyses remained ununited. From Dawson's findings on The Wistar Institute rats, Todd felt that an environmental factor had produced a metabolic disturbance of which non-union of epiphyses was the only significant evidence present in these animals. This now seems to be a well-substantiated observation in view of the great mass of experimental and clinical work which has been published especially by Todd.

In later publications Dawson in 1934 ('34 b) showed lapsed union in the Bussey strain of hooded rats and in a wild strain of captive Norway rats. These observations led him to believe that in rats a hereditary factor is responsible for the condition. There is little doubt that further experimental work would settle the question.

Koch ('34) investigated animals with a different type of post-embryonal growth to see if a uniform pattern of epiphyseal union obtains no matter what the precise body proportions might be. He felt that a study of ungulates, born

with short trunks and long legs, would afford a significant comparison with the animals already reported, e.g., Primates and rats which are born with long trunks and short extremities. In his study of the European bison he found a striking similarity to the patterns already cited. Comparison of these various patterns are set down in table 3.

The entire subject has been reviewed and enlarged in a new study by Todd ('38), now in process of publication, on epiphyseal union in mammals with special reference to Rodentia. Studies on the appearance of ossification centers and their differential growth in mice have appeared by Green and Fekete ('33) and Meng ('34). Harman and Saffry ('34) investigated the appearance of ossification centers in the fore limb of the guinea pig. They pointed out the absence of sex differences, a finding which appears also in the maturation study here presented.

MATERIAL AND STUDY

In 1931 a colony of guinea pigs was started under the auspices of the Brush Foundation, care being taken to get unrelated animals. These animals were kept in separate cages to prevent inbreeding and to establish standard conditions of living environment. No breeding was permitted under 6 months of age and there was no cross breeding or inbreeding. The diet consisted of an ample ration of alfalfa hay, carrots, turnips, apples, lettuce and laboratory-grown sprouted oats. In all 127 pigs were studied of which 65 were males and 62 were females. There can be no question of the adequacy of housing, nutrition, and health of these animals. Dorsoventral and left lateral roentgenograms were made of each animal at birth, at 2-week intervals up to 8 weeks of age, and then at intervals of from 3 to 5 weeks until the age of $2\frac{1}{2}$ years. Animals were sacrificed at different ages. Their skeletons were macerated and again x-rayed. We have therefore macerated skeletons of animals of both sexes at all ages up to $2\frac{1}{2}$ years. The serial x-rays of each animal were kept separately and these as well as the x-rays of the macerated skeletons and the

skeletons themselves were assessed by each of three observers independently. For invaluable aid in these assessments I am indebted to Dr. George R. Krause and Dr. George G. Linn who both worked on this problem during their undergraduate years in the Medical School.

The assessments of all the x-rays for each sex were grouped in significant age periods to determine the average age of union. X-rays of the living were repeatedly checked against x-rays of macerated skeletons. The bones themselves were also assessed by actual inspection to make sure that those of the living were correctly assessed.

The readings of the x-rays fall into four phases. In the tables these readings are given as follows:

1. (-) which means that union has not started. A clear space between epiphysis and diaphysis is seen. This is relatively wide until shortly before closure begins when the space narrows and, in consequence of practical cessation of growth, the surfaces of both epiphysis and diaphysis become more densely outlined. So long as a clear space remains the reading is one of ununited epiphysis.

2. (b) which means beginning union. Union usually starts at the center of the diaphyso-epiphyseal plane and proceeds to the periphery. Any interruption of the intervening space marks beginning union even though the process takes some time to complete. In certain regions peculiarities occur. For example, at the distal end of the radius, union starts on the lateral side and progresses toward the ulna. At the head of the humerus union begins at the center of the diaphyso-epiphyseal plane through which it slowly penetrates. Union at this site starts early. It requires a long time to complete and in some specimens the site of the plane is never entirely obliterated. This resembles in part the observation on The Wistar Institute rats but in our series it involves the greater tuberosity; it is never extensive and never occurs in the head of the femur in animals whose constitution shows the effect of balanced diet and proper living conditions. The epiphyses in these sites and in the vertebral column as well, frequently

show delayed union or incomplete or 'lapsed' union when some dietary, infectious, or endocrine disturbance has occurred. Separate studies of these factors will follow the present writing.

3. (*r*) which means complete union with persistence of the line of closure. At this stage a single line replaces the two lines formerly seen before union occurred. The line persists for some time and may easily be misinterpreted by the inexperienced observer. In some regions, e.g., distal femur, distal ulna, tibial tuberosity, calcaneus, metacarpals and metatarsals, the line remains for a long time. Checking against macerated skeletons gives the confidence necessary for positive assessment of even these areas.

4. (+) which means complete union without presence of the line of closure. There is now no sign of the diaphyso-epiphyseal plane, and the continuity of the bone is unbroken.

Since very little disparity in the ratings of the two sexes appeared, the sexes were grouped together in the final tables.

In table 1 the values given were computed from all the readings of each epiphysis. The ages in weeks accepted as characteristic of each phase are based upon the fact that more than half the animals in each group achieved the stated degree of union, i.e., beginning, recent, or complete. The findings showed no significant difference between male and female, maturity being a feature of the growing animal and not a sex-linked character. Table 1 gives their composite records. Figure 1 represents the same material graphically for ready reference.

The method of evaluation employed here has been used in recording epiphyseal progress in man and some of the anthropoids, but heretofore has not been applied to rodents. With a little experience it affords a means of investigation with a minimum of control animals and makes unnecessary the sacrifice of the experimental animals. The total number of estimations in this series is therefore actually much greater than in any similar preceding study.

In table 1 it will be noted that the proximal end of the fibula has not completely united in our oldest animals. At the proximal end of the humerus a distinction must be made between the epiphysis of the head and that of the greater tuberosity. Likewise at the upper end of the tibia distinction must be made between the principal epiphysis and that for the tubercle.

TABLE 1

Age order of epiphyseal union in the guinea pig in weeks:
b, beginning union; r, recent union; +, complete union

<i>Epiphysis</i>	<i>Age at union</i>		
	<i>b</i>	<i>r</i>	<i>+</i>
1. Distal humerus	9	10	14-15
2. Pelvis, primary elements	9	12	16-17
3. Middle phalanges, hand and foot	12	16-17	18-19
4. Proximal femur	12	18-19	20-21
5. 2nd metatarsal	14-15	20-21	23-24
6. Distal tibia	14-15	20-21	28-30
7. Proximal phalanges, hand and foot	16-17	20-21	22
8. Proximal radius	16-17	20-21	23-24
9. Calcaneus	16-17	20-21	25-27
10. Humerus head	16-17	28-30	37-40
11. Metacarpals 1, 2, 3	18-19	22	25-27
12. Metatarsals 1, 3	18-19	23-24	28-30
13. Distal femur	18-19	28-30	34-36
14. Metacarpal 4	22	25-27	28-30
15. Distal fibula	28-30	34-36	41-44
16. Humerus tuberosity	34-36	49-52	61-68
17. Proximal ulna	37-40	49-52	61-68
18. Acromion	37-40	76-80	116-124
19. Proximal tibia	41-44	69-75	101-108
20. Distal radius	49-52	61-68	86-96
21. Distal ulna	61-68	76-86	101-108
22. Tibial tubercle	69-75	101-108	116-124
23. Proximal fibula	85-95	116-124	

Long after union is complete for the head of the humerus and for the proximal tibial epiphysis there is still a small gap between the shaft of the humerus and the epiphysis for the great tuberosity and a gap between the shaft of the tibia and the epiphysis for the tubercle of the tibia.

The ossification of guinea pig epiphyses at birth roughly corresponds with that of man at 8 years; by 8 weeks the guinea

pig epiphyses have reached the stage of development of those in man at 12 years. But it is not until the guinea pig is 80 weeks old that its epiphyses correspond roughly to those of man at 17 years.

In table 2 are the dates of union for the epiphyses of the vertebral bodies, the dates for the extent of ossification of the iliac crest and date for the union of the epiphyses of the

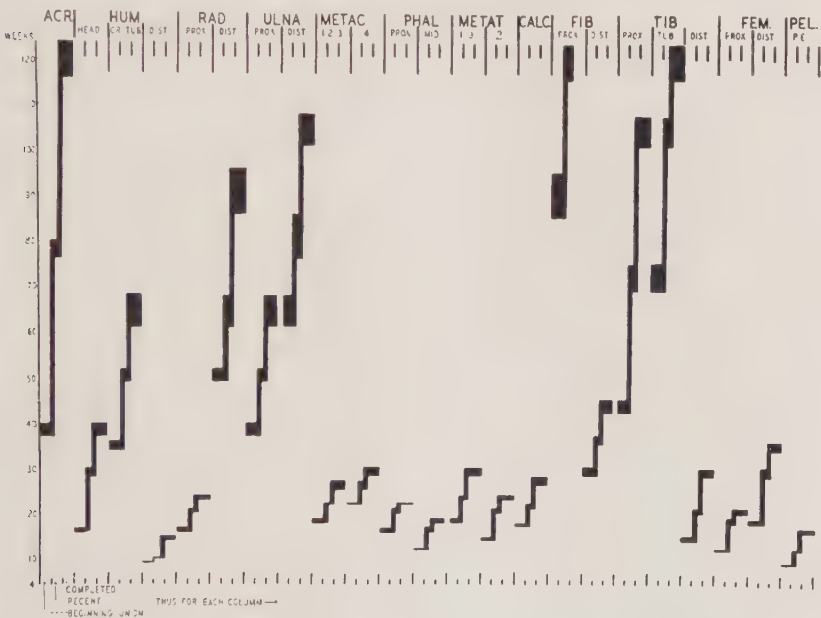


Fig. 1 Epiphyseal union is represented in three phases. Each heavy black line has three steps, the lowest representing beginning union, the middle one, recent union, and the uppermost, complete union.

vertebral border and angle of the scapula. Clear x-rays permit accurate assessment of epiphyses of vertebral column even though there is overlapping of the shadows for epiphysis and body.

It is not until between 25 and 30 weeks that the guinea pig reaches an epiphyseal pattern comparable with that indicating fertility in woman. However, the guinea pig can be bred successfully at a much younger age, which means that there

is an epiphyseal picture uniform for all mammals to proclaim the advent of fertility. In other words, skeletal maturation and gonadal maturity are not directly related the one to the other but are both influenced by a common governing factor.

It is quite evident that growth has nearly stopped long before complete union of the epiphyses occurs. Linear measurements of a representative number of long bones have been

TABLE 2
Age order of epiphyseal union in the guinea pig in weeks

	b	+
C. vert., prox. ep.	14-15	25
C. vert., dist. ep.	16-17	25
T. vert., prox. ep. T 1-6	16-17	45
T 7-13	20-21	45
T. vert., dist. ep. T 1-3	20-21	45
T 4-7	31-33	45
T 8-11	41-45	49- 52
T 11-13	49-56	67- 72
L. vert., prox. ep. L 1-3	20-21	34- 36
L 4-5	25-27	34- 36
L. vert., dist. ep. L 1-3	34-36	85- 95
L 4-5	85-95	116-124
Iliac crest	53-60	100-108
$\frac{1}{3}$ ossified, 16-17		
$\frac{1}{2}$ ossified, 20-21		
$\frac{3}{4}$ ossified, 25-27		
all ossified, 28-30		
Scapular border, inf. angle	101-108	
$\frac{1}{3}$ ossified, 16-17		
$\frac{1}{2}$ ossified, 18-19		
$\frac{3}{4}$ ossified, 32-33		
all ossified, 37-40		

made and will be reported in a subsequent paper. It is important to emphasize the distinction between the rodent and the primate. In the latter, epiphyseal union takes place very shortly after cessation of growth.

Green and Fekete noted that in mice ('33) and Koch observed that in the bison ('34) metacarpals and metatarsals stop growing early but that their epiphyses remain ununited for a long time. This is true also of the guinea pig.

In table 3 a comparison of the findings by Stevenson, Dawson, Koch and ourselves is set down to show the practical uniformity of pattern in spite of wide zoölogical differences. The following exceptions from the usual mammalian order of union should be noted. There is early union of the head of the

TABLE 3
Comparison of order of union

Group I			
<i>Man</i> (<i>Stevenson</i>)	<i>Bison</i> (<i>Koch</i>)	<i>Rat</i> (<i>Dawson</i>)	<i>Guinea pig</i> (<i>Zuck</i>)
1. Dist. hum.	Dist. hum.	Dist. hum.	Dist. hum.
2. Med. ep. hum.	Med. ep. hum.	Prox. rad.	
3. Os innom.			Os innom.
4. Head rad.	Dist. tib.	Dist. tib.	Head fem.
5. Olecranon	Prox. rad.	Dist. fib.	Dist. tib.
6. Head fem.	Olecranon	Med. ep. hum.	Prox. rad.
7.	Calcaneus		Calcaneus
8. Dist. tib.	Head fem.	Olecranon	Head hum.
9. Dist. fib.	Dist. fib.	Head fem.	Dist. fem.
10. Prox. tib.	Prox. tib.	Dist. fem.	Dist. fib.
11. Prox. fib.	Prox. fib.	Prox. tib.	Olecranon
12. Dist. fem.	Dist. fem.	Prox. fib.	Prox. tib.
13. Dist. rad.	Dist. rad.	Dist. rad.	Dist. rad.
14. Dist. ulna	Dist. ulna	Dist. ulna	Dist. ulna
15. Head hum.	Head hum.	Head hum.	Prox. fib.
Group II			
Vert. col.	Sacrum	Ribs	Vert. col.
Scap. border	Vert. col.	Sternum	Scap. border
Acromion	Pelvis	Pelvic girdle	Acromion
Clavicle	Ribs	Pect. girdle	Iliac crest
Iliac crest	Scapula		Ischial tuber.

femur in the guinea pig whereas this epiphysis unites relatively late in man. The proximal ulnar epiphysis in the guinea pig unites relatively late whereas the humeral head unites relatively early. In these particulars the guinea pig also differs from the rat though in all other features the pattern of union in the two animals is practically uniform.

The epiphyseal union pattern reported in this study may serve as a standard for the quantitative assessment of experimental modification of growth and development in the guinea pig, resulting either from cross breeding or production of endocrine imbalance, modifications which may result in a permanent constitutional change.

In conclusion I wish to express my indebtedness to Dr. Wingate Todd who has permitted me full access to his records of epiphyseal union in Mammalia, especially those made upon Rodentia, and has allowed me to consult his manuscript on the subject. To Miss Kuenzel, Miss Lane, Mr. Arnold and others of my colleagues, whose material help and experience has served to amplify my own and to complete the preparation of the data, I owe a debt which is a pleasure to acknowledge.

SUMMARY

1. A table of epiphyseal union in the guinea pig is presented as a register of developmental age of the animal up to more than 2 years.

2. Sex differences in the epiphyseal pattern of the guinea pig are negligible.

3. The time relationships in the order of epiphyseal union are not uniform for mammals. Thus the pattern in the guinea pig at birth is the equivalent of 8 human years, and the pattern at 8 weeks is the equivalent of that in a 12-year-old child. But it is not until 80 weeks that the guinea pig pattern is the equivalent of 17 human years.

4. The maturity pattern of the guinea pig contrasts sharply with that of the rat in the order of union of some epiphyses of the superior extremity; and with that of man in the order of union of some epiphyses of the inferior extremity.

5. Effects on skeletal maturity of endocrine and breeding experiments in the guinea pig can now be accurately assessed.

LITERATURE CITED

- DAWSON, A. B. 1925 The age order of epiphyseal union in the long bones of the albino rat. *Anat. Rec.*, vol. 31, pp. 1-17.
- 1927 Further studies on the epiphyses of the albino rat skeleton with special reference to the vertebral column, ribs, sternum and girdles. *Anat. Rec.*, vol. 34, pp. 351-363.
- 1929 A histological study of the persisting cartilage plates in retarded or lapsed epiphyseal union in the albino rat. *Anat. Rec.*, vol. 43, pp. 109-129.
- 1934 a Further studies on epiphyseal union in the skeleton of the rat. *Anat. Rec.*, vol. 60, pp. 83-86.
- 1934 b Additional evidence of the failure of epiphyseal union in the skeleton of the rat. Studies on wild and captive gray Norway rats. *Anat. Rec.*, vol. 60, pp. 501-511.
- GREEN, C. V., AND E. FEKETE 1933 Differential growth in the mouse. *J. Exp. Zool.*, vol. 66, pp. 351-370.
- HARMAN, M. T., AND O. B. SAFFRY 1934 The skeletal development of the anterior limb of the guinea pig, *Cavia cobaya* Cuv., from the 25-day embryo to the 161-day postnatal guinea pig. *Am. J. Anat.*, vol. 54, pp. 315-331.
- HENLE, J. 1871 *Handbuch der systematischen Anatomie des Menschen*. I. *Handbuch der Knochenlehre*, 3te Aufl. Friedrich Vieveg u. Sohn, Braunschweig.
- KOCH, W. 1934 The age order of epiphyseal union in the skeleton of the European bison (*Bos bonasus* L.). *Anat. Rec.*, vol. 61, pp. 371-376.
- MENG, T. H. 1934 The time and order of appearance of ossification centers in *Mus musculus*. *Peking Nat. Hist. Bull.*, vol. 9, pp. 7-14.
- STEVENSON, P. H. 1924 Age order of epiphyseal union in man. *Am. J. Phys. Anthropol.*, vol. 7, pp. 53-93.
- TODD, T. W. 1930 a The anatomical features of epiphyseal union. *Child Develop.*, vol. 1, pp. 186-194.
- 1930 b The roentgenographic appraisalment of skeletal differentiation. *Child Develop.*, vol. 1, pp. 298-310.
- 1931 Differential skeletal maturation in relation to sex, race, variability and disease. *Child Develop.*, vol. 2, pp. 49-65.
- 1933 Human bodies and human beings. *Sigma Xi Quart.*, vol. 21, pp. 123-140.
- 1937 *Atlas of skeletal maturation*. Part I. The hand. The C. V. Mosby Co., St. Louis.
- 1938 Epiphyseal union in mammals with special reference to Rodentia. In publication.

HYPERPLASIA AND HYPERTROPHY OF THE UTERINE MUSCULATURE IN OVARIECTOMIZED RATS FOLLOWING ESTRONE INJECTIONS

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ONE PLATE (SEVEN FIGURES)

INTRODUCTION

Various workers have observed an increase in the amount of the uterine musculature following estrone injections, but it has not been determined whether this increase is due to hypertrophy, hyperplasia, or both.

Andrie ('24) reported hyperplasia of the uterine muscle fibers in the estrous rabbit and Anapolsky ('28) found an increase in the length of the tubal muscle fibers of the sow during heat. Newton ('33) found that the most obvious change in the uterus of the guinea pig after estrone injections was an enlargement which he stated might be associated with an increase in the size, number or both of the muscle fibers.

Freud ('33), Overholser and Nelson ('34, '35) reported that estrone caused hypertrophy of the seminal vesicle musculature in rats. David, Freud and de Jongh ('34) found that castrated female rabbits and rats, when injected with estrone daily for several weeks, developed very thick uteri with a narrow unramified cavity. The thickened wall consisted for the most part of enormously hypertrophied submucous or interstitial tissue, reminiscent of fibroid tumors.

Robinson and Langston ('35) reported the effect of estrone on atrophic uteri of castrated rats. They found estrone to restore the horns to practically their normal diameters, after

having passed through an atrophic stage of definitely reduced diameters.

Robson ('35) found that injections of estrone in the hypophysectomized rabbit produced alterations similar to those observed in the ovariectomized rabbit. He found hypertrophy involving both the endometrium and muscle of the uterus.

That estrone causes a growth in the musculature of the uterus has been observed. It has not been shown whether this increase in musculature is due to hypertrophy, hyperplasia or both. This fact has led us to investigate the problem.

MATERIAL AND METHODS

Fourteen experimental groups consisting of a total number of seventy-three rats were used. These consisted of thirty-eight injected castrated animals, seventeen uninjected castrated controls, eleven normal animals sacrificed at time litter mates were castrated, and seven unoperated and uninjected normal controls. Thirteen of these groups consisted of litter mate female albino rats of known ages, the remaining group consisting of adult rats whose ages were unknown. In each group some of the animals were used as injected castrates, others as uninjected castrate controls; most groups had normal unoperated controls, and some groups had as further controls animals which were sacrificed at the time of castration of their litter mates.

The ages of the groups at date of castration varied from 27 to 77 days, one exception being the unknown age group. The weights of the animals were obtained at the time of castration, again when injections of estrone were begun, and finally when the animals were sacrificed at the end of the injection period.

In order to get variations in the atrophic condition of the uteri after castration, the period between the time of castration and the starting of estrone injections was set at three different time periods: 1 month, 2 months and 3 months.

The following four dosages of estrone¹ were used: 5 rat units injected daily for 15 days; 10 rat units injected daily for 10 days; 15 rat units injected daily for 10 days; 25 rat units injected daily for 30 days. All injections of estrone were made subcutaneously in oil solution.

The animals were killed with ether, weighed and autopsied according to a routine procedure similar for all animals. All uteri were removed at the same time interval after death of the animals, and were immediately measured and then placed in Bouin's solution. The tissues were run through the ordinary paraffin technique, sectioned at 10 μ and stained with Mallory's triple stain. In the procedure of preparing the tissue for histological study, care was taken to see that all tissues were subjected to the same treatment.

Camera lucida drawings were made of similar sections of both uterine horns and body of each uterus. Measurements of the total muscular thickness were made directly from the section by means of an ocular micrometer. The average of five micrometer readings, taken for any one transverse section, was recorded in each case. After obtaining the total muscular thickness directly by means of the ocular micrometer, we were able to compute the thicknesses of the longitudinal and circular muscle layers on obtaining their ratio to each other from measurements made on the camera lucida drawings. This procedure was to determine whether or not estrone caused an increase in the musculature of the uteri and if so, whether it was greater in the circular or the longitudinal layer. After observing an increase in the musculature produced by the injection of estrone a detailed study of the muscle fibers was made in order to determine whether an hypertrophy of the fibers had occurred.

To determine whether or not there was an hyperplasia of the muscle fibers, transverse sections of the uterine horns were divided into quarters by ink lines marked on the cover slip. The total number of longitudinal muscle fibers in one-quarter of the section was counted and recorded. The area

¹We are indebted to Dr. Oliver Kamm, of Parke, Davis and Company, for supplying the Theelin used in this work.

used for counting muscle fibers was the upper fourth of the circumference opposite the thinner longitudinal muscle layer caused by an accumulation of vessels. Thus the same region was compared in the experimental animals and controls.

PRESENTATION OF DATA

The effect of estrone injections on the size of the uterus and thickness of the muscle is summarized in table 1. In order to simplify the table only the average figures for each group are given. At the bottom of the table an average of the group averages is taken. The figures for the normal unoperated controls and controls sacrificed at the time of castration of experimental animals are also omitted for purposes of simplifying the table. Variations in the estrone dosage, length of time of injection and castration period produced no significant differences. The figures clearly show, however, that the estrone injected castrated animals had larger uteri (horns, 14.6×2.8 mm.; body, 6.3×4.6 mm.) than the castrated control animals (horns, 10.8×1.7 mm.; body, 3.9×2.7 mm.). This increase in size of the castrate uterus following estrone injections is due, in part, to an increase in the musculature (figs. 1, 2, 3, 4, 5). The total muscle thickness in the injected castrated animals was 0.4308 mm. while in the castrated control animals it was 0.1969 mm. The circular layer apparently underwent a greater increase (0.1180 to 0.2792 mm.) than the longitudinal layer (0.0800 to 0.1509).

A careful comparison of the sections from estrone injected animals with sections from the castrate controls showed that the cross sectional areas of the individual muscle fibers in the longitudinal layer were larger in the estrone injected animals than in the castrate controls (figs. 6, 7). This study of the sections clearly showed that an hypertrophy of muscle fibers had occurred in castrated animals as a result of estrone injections.

The effect of estrone injections on the number of muscle fibers is shown in table 2. The average number of fibers per counting area for the injected animals was 2121 and for the castrate controls was 1040.

TABLE 1

Effect of estrone injections on size of uterus and thickness of muscle in castrated rats

INJECTED ANIMALS				AVERAGE UTERINE MEASUREMENTS, MM.				AVERAGE THICKNESS OF MUSCLE IN HORNS, MM.				CASTRATE CONTROLS			
Number of animals	Age at castration	Dosage in rat units and days injected	Average body weight at sacrifice	Injected animals		Castrate controls		Injected animals		Castrate controls		Average body weight at sacrifice	Number of animals		
				Horns	Body	Horns	Body	Total thickness	Longitudinal layer	Circular layer	Total thickness			Longitudinal layer	Circular layer
Animals castrated for 1 month															
4	days	5 × 15	gm.	16.0 × 2.7	6.0 × 4.4	11.0 × 2.0	4.5 × 2.7	0.3579	0.1417	0.2162	0.2419	0.0941	0.1477	gm.	2
3	77	10 × 10	151	14.6 × 3.1	6.1 × 4.3	13.5 × 1.5	5.5 × 3.0	0.4166	0.1690	0.2475	0.1845	0.1009	0.0830	150	1
3	75	10 × 10	159	16.0 × 3.0	6.5 × 5.0	12.2 × 2.0	4.5 × 3.0	0.4469	0.1711	0.2757	0.1968	0.0768	0.1200	165	1
3	72	15 × 10	153	14.6 × 3.0	6.5 × 5.0	15.5 × 1.5	4.5 × 2.5	0.4319	0.1666	0.2671	0.2378	0.1184	0.1193	155	1
3	64	15 × 10	123	13.0 × 2.3	6.6 × 4.3	11.0 × 2.0	4.5 × 3.0	0.3963	0.1469	0.2492	0.1804	0.0675	0.1129	130	1
Animals castrated for 2 months															
2	54	5 × 15	195	14.5 × 3.4	5.5 × 5.0	7.5 × 1.5	2.5 × 2.5	0.5216	0.1960	0.3206	0.2214	0.1085	0.1128	200	1
4	75	10 × 10	186	20.2 × 2.3	6.2 × 4.0	6.5 × 1.5	2.5 × 2.5	0.3772	0.1348	0.2424	0.2102	0.1064	0.1207	200	1
Animals castrated for 3 months															
2	..	5 × 15	225	14.7 × 3.7	7.0 × 5.0	12.0 × 2.2	4.0 × 3.0	0.6150	0.2192	0.3957	0.3854	0.1582	0.2271	235	1
2	48	5 × 15	225	11.5 × 2.5	5.5 × 4.0	9.2 × 1.4	3.0 × 2.8	0.3936	0.1304	0.2632	0.1640	0.0658	0.0981	235	1
2	66	10 × 10	220	16.7 × 2.8	6.7 × 4.7	15.5 × 1.6	4.0 × 2.6	0.4182	0.1522	0.2657	0.1640	0.0607	0.1033	212	2
3	27	10 × 10	213	12.0 × 2.9	6.3 × 5.0	7.5 × 1.5	3.0 × 2.5	0.3471	0.1034	0.2421	0.1558	0.0589	0.0968	215	1
2	65	25 × 30	162	13.2 × 3.2	6.2 × 5.2	9.5 × 1.5	3.0 × 1.5	0.4428	0.1257	0.3171	0.1148	0.0328	0.0820	210	1
3	37	25 × 30	188	13.4 × 2.8	6.5 × 4.5	10.0 × 1.6	4.5 × 2.5	0.4271	0.1366	0.2897	0.1366	0.0382	0.0984	205	2
2	38	25 × 30	196	13.7 × 2.2	6.0 × 4.5	10.5 × 1.5	5.0 × 3.0	0.4400	0.1202	0.3170	0.1640	0.0328	0.1312	195	1
38		Average	181	14.6 × 2.8	6.3 × 4.6	10.8 × 1.7	3.9 × 2.7	0.4308	0.1509	0.2792	0.1969	0.0800	0.1180	189	17

TABLE 2

Effect of estrone injections on the number of muscle fibers in one-fourth of a section area of the longitudinal muscle layer of the uterine horns in castrated rats

INJECTED ANIMALS					CASTRATE CONTROLS		
Animal no.	Age at castration	Dosage in rat units and days injected	Uterine horn	Number of fibers	Number of fibers	Uterine horn	Animal no.
Animals castrated for 1 month							
13	75	10 × 10	Left	2430	1705	Left	16
14	75	10 × 10	Right	3932	1620	Right	16
15	75	10 × 10	Right	2700			..
19	72	15 × 10	Left	1810	1634	Left	21
20	72	15 × 10	Left	2234	1322	Right	21
20	72	15 × 10	Right	1503			..
25	64	15 × 10	Left	2642	1707	Left	28
26	64	15 × 10	Right	1399	1074	Right	28
27	64	15 × 10	Left	2568			..
27	64	15 × 10	Right	2703			..
Animals castrated for 2 months							
31	54	5 × 15	Left	2222	862	Left	33
31	54	5 × 15	Right	1945	876	Right	33
32	54	5 × 15	Left	1864			..
32	54	5 × 15	Right	3409			..
Animals castrated for 3 months							
45	48	5 × 15	Left	1865	824	Left	47
45	48	5 × 15	Right	2759	1048	Right	47
46	48	5 × 15	Left	1632			..
46	48	5 × 15	Right	1564			..
50	66	10 × 10	Left	1865	1017	Left	51
50	66	10 × 10	Right	2035	816	Right	51
..	..				854	Left	52
..	..				621	Right	52
55	27	10 × 10	Left	1596	1219	Left	58
56	27	10 × 10	Left	1705			..
57	27	10 × 10	Left	1714			..
61	65	25 × 30	Right	1441	679	Right	62
61	65	25 × 30	Left	1353			..
64	37	25 × 30	Left	2479	640	Left	66
64	37	25 × 30	Right	2650	750	Right	67
65	37	25 × 30	Left	2809			..
65	37	25 × 30	Right	1232			..
70	38	25 × 30	Right	1588	500	Right	72
Average				2121	1040		

DISCUSSION

The above experiments clearly show that estrone stimulated the growth of the uterine musculature in castrated rats. The increase in the thickness of the muscle occurred in both the longitudinal and circular layers but was greater in the latter. This result conforms with that of Markee, Wells and Hinsey ('36) who found that the increased growth of the musculature of the rabbit uterus, due to distention, was greater in the circular layer.

A histological study of the cross section size of the individual muscle fibers in the longitudinal layer showed that the fibers were consistently larger in diameter in the estrone injected animals than in the castrate controls (figs. 6, 7). In determining whether hyperplasia of the muscle fibers had also occurred we limited the counting of the fibers to similar fourths of sections of the longitudinal muscle layer. Counts in the injected animals ranged from 1232 to 3932 with an average of 2121 fibers, while in the castrate controls the counts ranged from 500 to 1707, with an average of 1040 fibers. These figures definitely show that estrone caused an hyperplasia of the muscle fibers.

Variations in the estrone dosage produced no significant differences. A dosage of 5 rat units of estrone for 15 days was as effective as 25 rat units for 30 days. Neither did variation in the castration period of atrophy above 1 month modify the effects of the estrone.

The stimulation of uterine muscle growth by estrone probably occurs in pregnancy and may be a causative factor in the development of fibro-myoma of the uterus as postulated by Witherspoon ('35). He states as follows:

A distinction should be made between uterine hypertrophy and hyperplasia and true fibroid formation. In hypertrophy the etiological factor is an abnormally high concentration of the estrogenic principle in the circulation, acting apparently on normal uterine muscular tissue. In true tumor formation, on the other hand, as in fibroids, the increased ovarian follicular hormone in the blood is acting upon a hyper-susceptible tissue which has the capacity to concentrate this hormone at the site of the tumor.

Nelson ('37) has reported the development of fibromyomatous growths in the uterus of the guinea pig following prolonged administration of estrone.

SUMMARY

1. Daily subcutaneous injections of estrone were made in rats ranging in age from 80 to 156 days after castration periods of 1, 2 and 3 months. Four dosages of estrone were used: 1) 5 rat units for 15 days; 2) 10 rat units for 10 days; 3) 15 rat units for 10 days; 4) 25 rat units for 30 days.

2. Estrone caused a definite increase in the length and diameter of the horns and bodies of the uteri.

3. Estrone caused a marked increase in the thickness of the uterine musculature. The increase in thickness was greater in the circular muscle layer than in the longitudinal layer.

4. Estrone produced an hypertrophy of the individual muscle cells.

5. Estrone produced an hyperplasia of the muscle cells.

6. Variations in the estrone dosage and time of castration atrophy did not result in any significant differences.

LITERATURE CITED

- ANDREI, O. 1924 Hyperplasie du tissu musculaire de l'uterus chez les lapines en rut. *Arch. Ital. de Biol.*, T. 73, pp. 52-54.
- ANOPOLSKY, D. 1928 Cyclic changes in the size of muscle fibers of the fallopian tube of the sow. *Am. J. Anat.*, vol. 40, pp. 459-469.
- DAVID, K., J. FREUD AND S. E. DE JONGH 1934 Conditions of hypertrophy of seminal vesicles in rats. II. The effect of derivatives of estrone (menformon). *Biochem. J.*, vol. 28, pp. 1360-1367.
- FREUD, J. 1933 Conditions of hypertrophy of the seminal vesicles in rats. *Biochem. J.*, vol. 27, pp. 1438-1450.
- MARKEE, J. E., W. M. WELLS AND J. C. HINSEY 1936 Studies on uterine growth. III. A local factor in the rabbit uterus. *Anat. Rec.*, vol. 64, pp. 221-230.
- NELSON, W. O. 1937 Endometrial and myometrial changes, including fibromyomatous nodules, induced in the uterus of the guinea pig by the prolonged administration of oestrogenic hormone. *Anat. Rec.*, vol. 68, pp. 99-102.
- NEWTON, W. H. 1933 The normal behavior of the isolated uterus of the guinea pig, and its reactions to oestrin and oxytocin. *J. Physiol.*, vol. 79, pp. 301-316.

- OVERHOLSER, M. D., AND W. O. NELSON 1934 The effect of estrin administration on the seminal vesicle musculature of castrated rats. *Anat. Rec.*, vol. 58 (supplement), pp. 31-32.
- 1935 The effect of estrin and male hormone injected separately and simultaneously upon the smooth muscle and epithelium of the seminal vesicle in the albino rat. *Anat. Rec.*, vol. 62, pp. 247-267.
- ROBINSON, B. L., AND W. C. LANGSTON 1935 Castration atrophy and theelin. Effect of theelin on atrophic uteri of castrated albino rats. *Endocrinology*, vol. 19, pp. 441-446.
- ROBSON, J. M. 1935 The action of oestrin on the uterus of the hypophysectomized and of the pregnant rabbit. *J. Physiol.*, vol. 84, pp. 148-161.
- WITHERSPOON, J. T. 1935 The hormonal origin of uterine fibroids. An hypothesis. *Am. J. Cancer*, vol. 24, pp. 402-406.

PLATE 1

EXPLANATION OF FIGURES

1 Section through left uterine horn of uninjected control 40 days after castration. $\times 57$.

2 Section through left uterine horn of animal that had received 10 rat units of estrone daily for 10 days after a castration period of 1 month. Marked increase in the thickness of the musculature as compared with litter mate uninjected control shown in figure 1. $\times 57$.

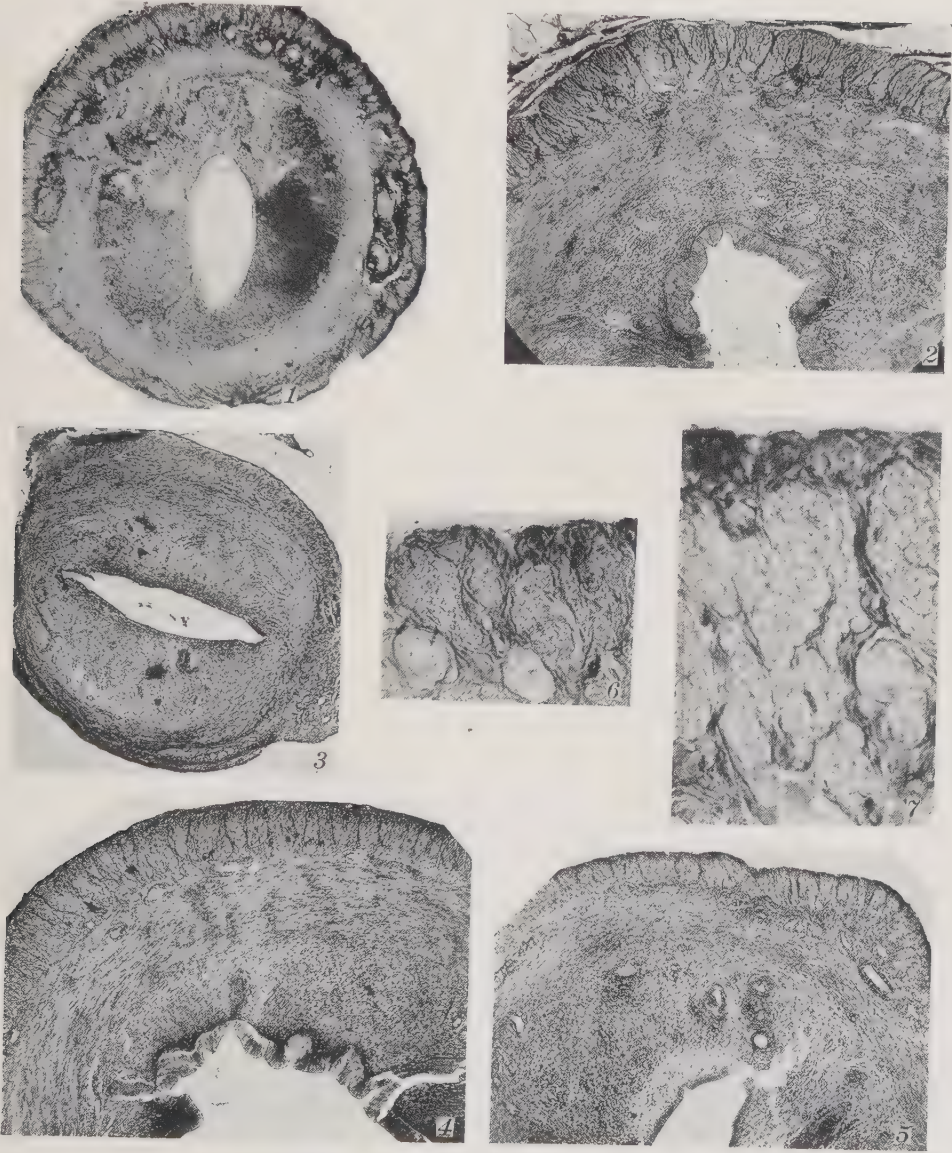
3 Section through the right uterine horn of uninjected control 4 months after castration. $\times 57$.

4 Section through right uterine horn of animal that had received 25 rat units of estrone daily for 30 days after a castration period of 3 months. Marked increase in thickness of the musculature as compared with uninjected litter mate control shown in figure 3. There is also an increase in the musculature as compared with the normal uninjected litter mate control (fig. 5). $\times 57$.

5 Section through the right uterine horn of normal uninjected control from same litter as animals shown in figures 3 and 4 and sacrificed at the same age. Normal thickness of muscle is not as great as in the castrated injected animal (fig. 4). $\times 57$.

6 Cross section of longitudinal muscle layer of uterine horn from uninjected control 40 days after castration. $\times 360$.

7 Cross section of longitudinal muscle layer of uterine horn from an animal that had received 15 rat units of estrone daily for 10 days after a castration period of 1 month. There is a marked increase in the size and number of the muscle fibers as compared with litter mate uninjected control shown in figure 6. $\times 360$.



A REVIEW OF THE GOLGI APPARATUS

PART I

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ONE PLATE (SEVENTEEN FIGURES)

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1. INTRODUCTION²

When Camillo Golgi in 1898–1899 described a complicated system of anastomosing, argentophilic strands forming a cytoplasmic reticulum in the Purkinje and the ventral horn cells of the barn owl, and around the nuclei of the spinal ganglion cells of the cat (fig. 1), he initiated a grand series of investigations, speculations, and controversies which even after 40 years has not ended. The literature of this subject has be-

¹ Parts II and III will appear, respectively, in the April and May issues of *The Anatomical Record*.

² The authors wish to express their appreciation to the numerous persons who have read and criticized the manuscript for this paper. In particular they thank Professor Beams for calling their attention to several papers which they had overlooked.

come so progressively unwieldy that it has been summarized periodically (Duesberg, '14; Cajal, '15; Pappenheimer, '16; Cowdry, '23, '24; Jacobs, '27; Bowen, '29; Hertwig, '29; MacBride and Hewer, '31; etc.). Several of the more recent reviews have ably summarized special aspects of the literature, but at the present time a new comprehensive survey is needed.

Since its discovery this 'apparato reticolare interno' of Golgi has been a constant challenge to cytologists. Many have denied validity to the challenge by claiming artificiality for the apparatus. Others have tried to homologize the reticulum with various known cytological constituents. A large group of investigators has confined itself to a study of the occurrence and form of the apparatus. Only within recent years has a concentrated attack been made upon the difficult problem of function.

To make a list of all the different types of cells in which the Golgi apparatus has been reported would require more space than the value of the list would warrant. It is simpler to say that the only cells in which it has not been possible to identify the structure are certain short-lived or dying ones, such as mammalian erythrocytes, cornified epithelial cells and most mature sperm cells, or cells which, because of their inaccessibility, or peculiar morphology, are particularly unfavorable for study, e.g., retinal rods and cones. Key ('32) declared that neither the Golgi apparatus nor its negative image had been demonstrated in any of the cells of synovial membranes, but King ('35) found no difficulty in demonstrating, in human synovial cells, well-developed Golgi apparatuses comparable with those of other connective tissue cells. Absence of the apparatus is not characteristic of all dying cells, for in many glands of the holocrine type it is recognizable in the glandular lumen as a part of the secretory mass even after the parent cell has lost its identity. Even in the protozoa there is a considerable body of evidence pointing toward the presence of a Golgi apparatus (Hall, '36). It is becoming increasingly probable that plant cells, too, possess a structure

identical with, or at least similar to, the internal reticular apparatus of animal cells (figs. 2, 8) (Gicklhorn, '32). Our knowledge of the apparatus in plant cells is reviewed by Zirkle ('37).

A few years ago students of the Golgi apparatus were perplexed by their failure to find any typical apparatus in tissue cultures of animal cells. Ludford ('27) attempted to explain the difficulty as follows: "As some cells spread out on the surface of the coverglass, the Golgi apparatus is stretched until it fragments, and its individual particles become dispersed in the cytoplasm eventually to such an extent, that the individual particles are below the limit of microscopical resolution." A few workers notably Chlopin ('24, '25) and Rumjantzew ('27, '29) described in tissue cultures what they called Golgi bodies, but in most instances it is doubtful whether the substances described represent the Golgi apparatus as seen in suitably fixed preparations of similar uncultured cells. Levi ('34) after briefly reviewing the subject concluded that "Der sog. Apparat von Golgi kann also demnach vacuolär, mitochondrio-vacuolär oder mitochondrial sein. Lassen wir also diesen Gegenstand, der unseres Erachtens zu jenem Teil der Cytologie gehört, der heute sowohl seines Inhaltes als auch der Methoden wegen, durch die derselbe untersucht wird, als überholt betrachtet werden kann, beiseite." Even recently Canti ('33) reported failure to find any Golgi apparatus in tissue cultures. Weier ('33), too, stated that "investigations of the Golgi zone in the field of tissue culture have yielded quite uniformly negative results." Others, however (Champy, '26; Ludford, '27, '35; Champy and Morita, '28; Zweibaum and Elkner, '30; López, '32; Saguchi, '33; Gatenby and Hill, '34; Richardson, '34; Hill, '36; Macdougald, '35, '37, and Macdougald and Gatenby, '35) described unmistakable Golgi apparatus in their preparations and Macdougald ('37) reviewed its presence in tissue cultured cells. Hill ('36), who found perfectly normal Golgi apparatuses in chick osteoblasts grown in vitro, attributed the failures of early workers to unsuitable conditions of cultivation.

At the present time two methods for demonstrating the Golgi apparatus are in general use (Bowen, '28); the silver impregnation methods, especially as employed by Cajal and Da Fano, and the osmication methods devised by Kopsch, Weigl and Kolatchev. The silver methods were most commonly employed by the earlier investigators, but most workers today show a preference for osmication methods, particularly for Nassonov's modification of the Kolatchev technique.

As numerous commentators have pointed out, it is unfortunate that, despite repeated attempts, few clear observations have been made of the Golgi reticulum in living cells. If the Golgi apparatus is "a differentiated, dynamic region of cytoplasm, homogeneous to the eye, but in reality crossed by irregular interfaces upon which osmium is precipitated," as suggested by Lillie and others and defended at some length by Weier, the apparent invisibility of the apparatus in the majority of living cells becomes readily comprehensible. But attractive as this conception is, few investigators have given it serious consideration.

Direct transmitted light (Baumgartner, '33), dark-field illumination (Lewis, '23; Strangeways and Canti, '27 [but see the convincing objections advanced by Macdougald and Gatenby, '35]), and ultraviolet light photography (Lucas and Stark, '31; Wyckoff and Ebeling, '33) have so far failed to disclose this apparatus with anything approaching the distinctness of a good osmium or silver impregnation. However, very little has yet been done with ultraviolet light; study of the apparatus by this means might prove well worth while. It is true that many authors claim to recognize the apparatus in living cells. von Bergen in 1904 appears to have been the first to do so, although according to Bowen ('28) and Douglas ('35), beginning with an historic paper of Dujardin's in 1837 a number of reports (notably by St. George, 1867; Platner, 1885, and Meves, '00) had previously been made of structures which in the light of present knowledge might have been Golgi apparatus. von Bergen gave no figures of living cells, however, and his description was not extensive. Other writers de-

scribed what they considered to be Golgi apparatus in an increasing variety of living, unstained and vitally stained cells. The structure described by these writers has in most cases been shown to be vacuome and not true Golgi apparatus. That these two cellular constituents are not the same has been definitely established by recent work of Beams and others. Other structures described, however, have undoubtedly been the reticulum itself. Successful staining of the apparatus with the vital dyes neutral red, Janus green, dahlia, gentian violet, methylene blue, cresyl blue, Nile blue sulphate, cresyl-echtviolet, Bismarck brown, thionin and Sudan III has been reported. A great variety of dyes has been used to stain the cell background, thereby producing a negative image of the apparatus. Of all these dyes the one most extensively used has been neutral red, but unfortunately most of the workers employing it have failed to discriminate between vacuome and Golgi apparatus. The results with Janus green, particularly those resulting from study of male germinal tissue, are probably more reliable; the Golgi materials (dictyosomes) being particularly favorable for study in spermatocytes and spermatids. It seems fairly certain that the so-called canalicular system observed repeatedly in living pancreatic islet cells (Bensley, '11; O'Leary, '30; Beams, '30) is the true Golgi apparatus. According to numerous recent claims Golgi apparatus, vacuome and mitochondria may be seen very easily and clearly in certain invertebrate and vertebrate oocytes some with, and some without, the aid of vital staining (Gatenby and Nath, '26; Bhattacharya and Das, '29; Gatenby, '29, '31 c; Krjukowa, '29; Gatenby and O'Brien, '30; Nath, '30, '31; Sembrat, '30; Wilson and Pollister, '37). Work done by Norminton ('37, under Gatenby's direction) indicated unmistakably that Gatenby and Nath had mistaken fat globules for Golgi bodies in oocytes of the earthworm; by means of the ultracentrifuge Norminton succeeded in stratifying into different layers, fat globules (Golgi bodies of Gatenby and Nath), rod-shaped Golgi bodies, and granular mitochondria, all of which she was also able to identify in living, unstained, non-

centrifuged material. At the same time that this paper threw doubt upon the reliability of some of the earlier observations it rendered still more certain the presence of Golgi bodies in living cells. The pioneer description by Champy ('26) and Champy and Morita ('28) of a Golgi network in unstained and supravitality stained germinal epithelial cells of rabbit ovary grown in tissue culture may also be accepted as representing genuine Golgi strands and not mitochondria or vacuome. Macdougald and Gatenby ('35) reported that the apparatus is visible in living mammalian Purkinje cells, spermatocytes, etc., and also in all insect and mollusc germ cells which they have examined. Ludford ('35) was not content with merely describing or drawing the apparatus in living cells but provided us with an excellent microphotograph of it in a living fibroblast grown in vitro. Hence the time-honored belief that Golgi materials cannot be seen in living cells is no longer pertinent. Observations by experienced and competent investigators leave no doubt but that the apparatus exists and can be demonstrated in both living and fixed protoplasm.

The most conclusive proof that the Golgi apparatus is an organelle is not the universality of its presence in properly fixed material, nor even the descriptions of it in living material, but rather its behavior as determined by microscopical observations of it in the same type of cell in different physiological conditions. This behavior is so characteristic and so constant that there should be no question concerning the reality of the substance or structure exhibiting it.

Those who are unconvinced will find it necessary to ignore the discovery by Beams and King ('34) that under the great centrifugal force developed by the ultracentrifuge the Golgi apparatus in living, uterine, gland cells becomes stratified, together with the rest of the protoplasm. Macdougald and Gatenby referred to this work as marking the end of the Golgi apparatus-artefact controversy.

2. STRUCTURE OF THE GOLGI APPARATUS

The Golgi apparatus appears to be the most protean of all cytoplasmic structures. It has been described as a fibrous reticulum, network, ring, or cylinder, a very irregular fenestrated plate, a more or less incomplete hollow sphere, vesicle, or cup, a collection of small spheres, rodlets and platelets or discs, a series of anastomosing canals, a group of vacuoles, and a differentiated region of homogeneous cytoplasm crossed by irregular interfaces.

At the present time relatively few investigators adhere to either the canalicular or the vacuolar concept. Golgi himself thought of the apparatus as being a fibrous network of constant occurrence in all cells. This point of view still finds favor with a few writers, mostly countrymen of Golgi (Monti, Migliavacca, Hosselet, Ross). There can be no doubt that the general form exhibited by the apparatus in the somatic cells of vertebrates is reticular, but this is far from true in the cells of invertebrates and in the germinal cells of vertebrates. Neither is the vertebrate type of Golgi reticulum always fibrous. Since the publications of Hirschler there has been a strong tendency among workers to look upon the apparatus as a fenestrated plate resembling a fibrous reticulum in optical sections. That the specific gravity of the apparatus is lower than that of the cytoplasm is evident from the effects of ultracentrifuging upon it (Beams and King, '34, '35; Beams, Gatenby and Muliyil, '36; Guyer and Claus, '36; Dornfeld, '36, '37; Hellbaum, '36). The work of Beams and King indicated a fluid or semi-fluid character of the apparatus since in ultracentrifuged uterine gland cells the Golgi material appears to stream through the cytoplasm. This observation was interpreted by Sawyer ('36) as demonstrating beyond question that the Golgi apparatus has no fixed morphological character. However, Brown ('36) and Hellbaum ('36) found that in spinal ganglion and thyroid cells the apparatus is displaced with very little distortion, suggesting a semi-solid nature. Recent studies by Guyer and Claus ('36) and Dornfeld ('37) strengthen the conclusions of Beams and King and

at the same time discredit the interpretation of Sawyer. According to these studies the Golgi apparatus, in ultracentrifuged anterior pituitary and adrenal cortical cells, is displaced in the form of a liquid drop, but later returns to a typical network in transplants of the centrifuged material.

The conception of the Golgi apparatus as a system of canals dates back to the observations of several investigators, chiefly Holmgren, soon after the discovery of the apparatus by Golgi. Holmgren considered the apparatus to be formed by the vacuolization and subsequent canalization of cytoplasmic processes penetrating into the cell from neighboring cells and believed by him to be nutritive in function. Within these nutritive canals Holmgren assumed the presence of a lipoidal substance which became coagulated by the action of certain fixatives and simulated a system of solid rods or filaments upon treatment with osmic acid. That fibrous processes from adjacent cells actually do enter the cytoplasm of certain invertebrate ganglion cells can scarcely be denied, but that intracellular canals originate as such living cytoplasmic structures is doubtful. Still more dubious is the idea that the canals correspond, either in origin, structure or position, to the apparatus described by Golgi (Beams and King, '32; Brown, '36).

Although the trophospongium theory has been universally discarded, the reader must not conclude that it rested upon a completely insecure foundation. Intracellular canals very similar to those which Holmgren described do exist and Holmgren's mistake was not so much in his actual observations as in the theoretical interpretations he placed upon them. Careful descriptions of intracellular canalicular systems communicating with intercellular spaces or ducts in the parietal cells of insect salivary glands and mammalian gastric glands have been published recently (Beams and King, '32 a, '32 b). But these systems appear to constitute a passageway for the discharge of secretory products of the cells rather than for the entrance of nutritive substances. But since Holmgren's intracellular, canalicular 'trophospongium' has been adequately reviewed and criticized by numerous experienced writers (Cajal,

Duesberg, Wilson, Cowdry, Bowen, Beams, Hertwig, etc.) its interest to cytologists is mainly historical.

Since the late nineteenth century such canalicular systems have been known to exist, but, except for the followers of Holmgren, no serious attempt to identify them with the Golgi apparatus had been made until 1924 when Nassonov suggested that the wall of the contractile vacuole of protozoa contained the homologue of the metazoan Golgi apparatus. Nassonov did not include intracellular canals in his studies, but, as pointed out by Beams and King, such canals may be considered homologous to contractile vacuoles from the point of view of both their morphology and physiology. The homology suggested by Nassonov was supported by MacLennan ('36), but denied by Lynch ('30), Hill ('33), Beams and King ('32 a, '32 b), Moore ('34) and King ('35). Beams and King clearly figured both canals and Golgi apparatus as independent structures within a single cell (fig. 5).

For many years R. R. Bensley supported the idea that the Golgi apparatus is homologous with the vacuolar system of plant cells (Bensley, '10; Owens and Bensley, '29). This theory was later defended at great length as the vacuome hypothesis by Guillermond, Parat, and their many pupils who, however, identified as vacuome only intracellular structures which stain intravitaly with neutral red; an identification challenged by Chang ('35), a student of Bensley. The voluminous literature which has accumulated during the past 10 years in support of this homology has been subjected recently to such destructive criticism that many former vacuome advocates have abandoned the theory. Bensley appears to have severed his affiliation with the vacuome school as indicated by his 1932 paper (p. 216), although he apparently is inclined to retain his original ('10, '11) conception of the Golgi apparatus as a canalicular system homologous with that of plants, as also do his associates, O'Leary ('30), Bartelmez and R. D. Bensley ('32) and Chang ('35). Cowdry ('32) still favors the conception of the neutral red vacuome as the real Golgi apparatus. Gatenby ('31 a) has given an excellent review of the

whole controversy. Bowen ('26 a, '27 a), Hirschler ('28), Beams and his associates ('30-'35), MacBride and Hewer ('31), Voinov ('34), Borghese ('35), Muliyl ('35) and Salazar ('36) also present very spirited arguments against the vacuome-Golgi apparatus homology. The work of Beams furnishes particularly crucial proof of the inaccuracy of the homology for he and his colleagues have demonstrated Golgi apparatus, vacuome, mitochondria and canals of Holmgren simultaneously within the same cell. It is enough to say here that the majority of authorities are now inclined to believe that the Golgi apparatus is neither canalicular nor vacuolar in character.

Since Golgi's first description of the reticular apparatus the view, that the apparatus is a compact substance neither canalicular nor vacuolar, has been defended by a large number of investigators, and particularly by Nassonov, Bowen, Gatenby and Ludford. Although Cajal in 1915 strongly implied the likelihood of such an association, Nassonov ('23 a) appears to have been the first to suggest that the Golgi apparatus is concerned with secretory processes. Nassonov arrived at this suggestion through the study of vertebrate glandular cells but also turned to the study of the contractile vacuole of protozoa for confirmation of his view. Cajal had already suggested a possible homology between the contractile vacuole of ciliates and the Golgi apparatus of vertebrates. Unlike the students of the vacuome, however, Nassonov never considered the vacuole to be of fundamental significance. Rather it was in the wall of the contractile vacuole that he found the substance to which he ascribed primary physiological importance in the production of secretory materials. He found this substance to respond to the usual osmic acid and silver nitrate techniques used for demonstrating the Golgi apparatus, i.e., it became definitely and intensely impregnated. Bensley's ('29) suggestion that such impregnated areas are to be explained on the basis of adsorption of reduced osmium or silver by suitable surfaces does not seem likely here; for the blackened structures described by Nassonov are often found separated

from the vacuolar membrane by a very appreciable distance; also they are sometimes in the form of rings or girdles about the vacuole (fig. 3). Following Nassonov's lead Bowen ('26) made an exhaustive study of the apparatus in glandular cells producing mucous, serous, lipoidal and watery secretions. In all of his papers he consistently described the apparatus as a solid structure, often very definitely related to vacuoles but never vacuolar itself. The same conclusion has been reached by so many workers in the field that there seems to be little reason for not giving it preference over all other views.

In considering the composition of the Golgi apparatus we find that the literature contains nothing of a satisfactorily definite character. Concerning this subject a considerable number of opinions has been expressed, but always with inadequate evidence. The majority of cytologists evince more or less faith in an essentially lipoidal apparatus. The sources of this faith may be attributed to the solubilities and to the osmiophilic properties of the Golgi substance, it being well established that unsaturated lipoids are blackened by osmic acid. However, as was pointed out as early as 1888 by R. Heidenhain, and re-emphasized in 1929 by Owens and Bensley, and also by Hoerr in 1936, everything which blackens with osmic acid is not lipoidal. Bensley stressed also the possibility that the blackening of the apparatus represents an adsorption phenomenon, the osmic acid having been previously reduced by the cell substrate. Attempts to demonstrate the Golgi apparatus with the aid of more specific tests for fats have not met with any outstanding success, although the results in a few isolated cases (Ciaccio, '10-'27; Cowdry, '12; Karpova, '25; Weiner, '26; Ma and Chang, '28) suggested that a really intensive investigation of the problem might establish the lipoidal character of part of the apparatus at least. All the evidence in favor of a lipoidal Golgi apparatus is not limited to reaction with osmic acid, however, for the solubility of the apparatus certainly resembles that of fatty substances. The fresh tissue must be fixed in watery fixatives and kept from contact with fat solvents until after the appa-

ratus has become thoroughly impregnated (Eastlick, '36). While this does not by any means prove the Golgi apparatus to be lipoidal, it strongly suggests it. Additional evidence that the apparatus is lipoidal is found in the fact that during ultracentrifuging it behaves as a lipoidal structure, for it goes to the centripetal pole of the cell (Beams and King, '34; etc.).

It has been suggested by several workers, especially Bowen ('20, '26 a, '28), that the Golgi system is of a duplex character, consisting not only of an osmiophilic, argentophilic, reticular system of lamellae, probably of lecithin-like character, but also of a chromophobic material of protein nature, the idiozome (Regaud, '10), Apparatinhalt (Hirschler, '18), idioendosome (Papanicolaou and Stockard, 18), Apparatin-ternum (Hirschler, '27), Golgi internum (Sembrat, '30). The existence of two such substances very intimately associated with each other in vertebrate male germ cells and invertebrate somatic cells has received abundant and undeniable confirmation. But Bowen's suggestion, that a protein substance similar to the germ cell idiozome may be a constant constituent of the Golgi apparatus wherever it occurs, still lacks sufficient evidence to warrant its acceptance as more than a working hypothesis although the suggestion is widely entertained, for MacBride and Hewer ('31, p. 108) wrote: "Although modified cytoplasm, known as idioplasm, is normally only found to accompany the apparatus in germ cells, it is also probably present in all cells, although not demonstrable." Gatenby ('31 a, p. 460) left no doubt in the reader's mind concerning his belief in the 'living lecithin-like lipoproteid' character of the material in the Golgi apparatus. Among other investigators favoring the view were Bowen ('24 a, '26 a), Kopsch ('26), Hirschler ('27) and Weier ('33). On the other hand, Voinov ('25), Jacobs ('27), Morelle ('27), Nassonova ('27), Weiner ('30) and others contend that the idiozome has no real significance. Uhlenhuth ('34) presents very interesting pictures of thyroid cells of the California newt in which this double character of the apparatus is well shown, but he remarks that "... one would often feel inclined to consider

the idiosomatic component as the true Golgi material on the surface of which the black particles have been adsorbed." Evidently he is inclined to disagree with those who believe in the duplex structure of the apparatus in vertebrate somatic cells and also with those who consider the real Golgi substance to be osmiophilic. This interpretation had already been developed in considerably more detail by Weier ('33) who looked upon the Golgi apparatus as a series of interfaces in a differentiated, dynamic, cytoplasmic zone. He suggested that this differentiated region of cytoplasm corresponds to the osmiophobic (idiozomatic) Golgi zone declaring that: "... all Golgi nets possess such an osmiophobic component and that it is this portion of the living Golgi zone which is probably most important." These suggestions that the idiozome is the true Golgi apparatus support the interpretation of Owens and Bensley. Although this represents at present a minority point of view, it is one which has much in its favor and should be given careful study.

As nearly as we have been able to determine it is this conception of an idiozomic component of the organelle which has given rise to the idea that it has a protein as well as a lipoidal constituent. But even the protein character of the idiozome is not to be accepted as a foregone conclusion for it, too, rests upon interpretations based upon only a few non-specific technical procedures. Poisson ('27), for example, succeeded in staining the idiozome with light green in Bouin-fixed spermatids of *Notonecta* according to a method for demonstrating one of the common connective tissue proteins, mucin. Papanicolaou and Stockard ('18), using guinea pig spermatocytes and spermatids, described a method by which the idiozome may be stained with acid fuchsin, a stain often used for demonstrating the proteins reticulin and collagen, but which, it must be remembered, will also stain certain lipoids.

Weigl ('10, '10 a) appears to have been the first to suggest that the Golgi apparatus is composed of two chemically different substances. His work is particularly interesting because it dealt with the classical apparatus of vertebrate somatic

cells, not with the idiozome of germ cells. He found that after bleaching the osmicated apparatus he could restain it with Weigert's resorcin-fuchsin, a widely employed stain for elastin in connective tissue. Kopsch ('26), after considerable experimentation of his own, confirmed Weigl's interpretation, but considered the protein constituent to be the trophosphonium. In the basophiles of the Champy-fixed guinea pig hypophysis (Kirkman, '37) the Golgi apparatus may be deeply stained with anilin blue (fig. 13), which is the collagenous fiber stain in several triple connective tissue stains, and, in agreement with the work of Weigl and Kopsch, the apparatus in osmicated preparations may be bleached and stained in the same manner. However, since the dyes are not specific for proteins, but may be made to color certain lipoids, and since osmic acid is not specific for lipoids, but may be made to blacken proteins, it is possible that the dye-staining portion of the apparatus is identical with the osmiophilic portion. If the bleached and restained apparatus does not coincide exactly with the unbleached osmicated apparatus it may be considered likely that the two techniques disclose chemically different portions, but if the pictures revealed by the two techniques do coincide exactly it may be considered likely that they have the same chemical structure. In the numerous descriptions of such bleached and restained preparations the importance of even slight variations in form or position from the original has been overlooked.

Beams and King ('32, '32 a) described and illustrated both osmiophilic and osmiophobic portions of the Golgi apparatus in grasshopper ganglion and salivary gland cells, but they ventured no opinion as to the chemical nature of the osmiophobic portion. Beams ('31) was unable to demonstrate a chromophobic constituent in the spinal ganglion cells of the rat, although he had previously ('30) suggested that the apparatus contains both lipoid and protein components in the pancreas. Neither has the great majority of other workers succeeded in demonstrating an unmistakable idiozomic substance in other vertebrate somatic cells. Nath ('33) believed

that "the osmiophilic part of the Golgi element consists of unsaturated lipoids and the osmiophobic part of very nearly fully saturated lipoids." It would seem, then, that the prevalent idea of a constant protein constituent of the Golgi apparatus still awaits confirmation. Cataphoresis experiments, performed upon suitable material, might supply valuable information concerning the possible colloidal nature of the apparatus. A review of the literature concerning the chemical and morphological duality of the apparatus was included in Hertwig's ('29) beautifully illustrated review.

Possible inorganic constituents of the Golgi apparatus have not been disclosed by any methods now available. In reference to the microincineration technique Scott ('33) wrote: "Golgi apparatus, vacuome and mitochondria do not seem to be differentiated from the remainder of the cytoplasm in that they show no inorganic salt residue following incineration." Similar findings were reported by MacLennan and Murer ('34).

The success with which Bensley and Gersh ('33) have employed their recently developed freezing-drying method in the direct chemical study of mitochondria inclines one to look hopefully toward their laboratories for more exact knowledge of the chemistry of the Golgi materials.

The apparatus may be present in a cell as a single organelle or as many discrete portions. In the former case it may present a more or less compact appearance typical of most resting cells (figs. 15, 16A), or it may possess a loose, widespread character as in many spinal ganglia (figs. 1, 14) and many glandular cells packed with secretion granules (figs. 6, 11, 12, 17C). The dispersed type of apparatus, consisting of many discrete portions, is characteristic of most invertebrate cells and of vertebrate germinal cells. The portions are ordinarily in the shape of crescents, discs, cups, plates, granules or rodlets and may be uniformly scattered throughout the cytoplasm, as in some egg cells (fig. 10), or they may be more or less concentrated in a limited portion of the cytoplasm, as in many spermatocytes and spermatids (fig. 4A). If the osmio-

philic platelets discovered by Bowen ('26 b, '27, '28 a, '30) in plant cells are really homologues of the Golgi apparatus of the animal cell as Patten, Scott and Gatenby ('28), Gatenby ('28) and Beams and King ('35) believed, the dispersed type is characteristic of plant cells also (fig. 2). The manner in which the compact variety of apparatus may be derived from the dispersed type is considered by Subramaniam and Gopala Aiyar ('36 a). Coupled with the extreme morphological variability of the Golgi apparatus in animal and plant cells is the marked constancy of form which it exhibits in a given type of cell, e.g., in the anterior pituitary gland of certain mammals the acidophilic and the basophilic cells may be readily distinguished from one another by this criterion alone (Atwell, '32; Severinghaus, '33) (fig. 16).

TABLE 1

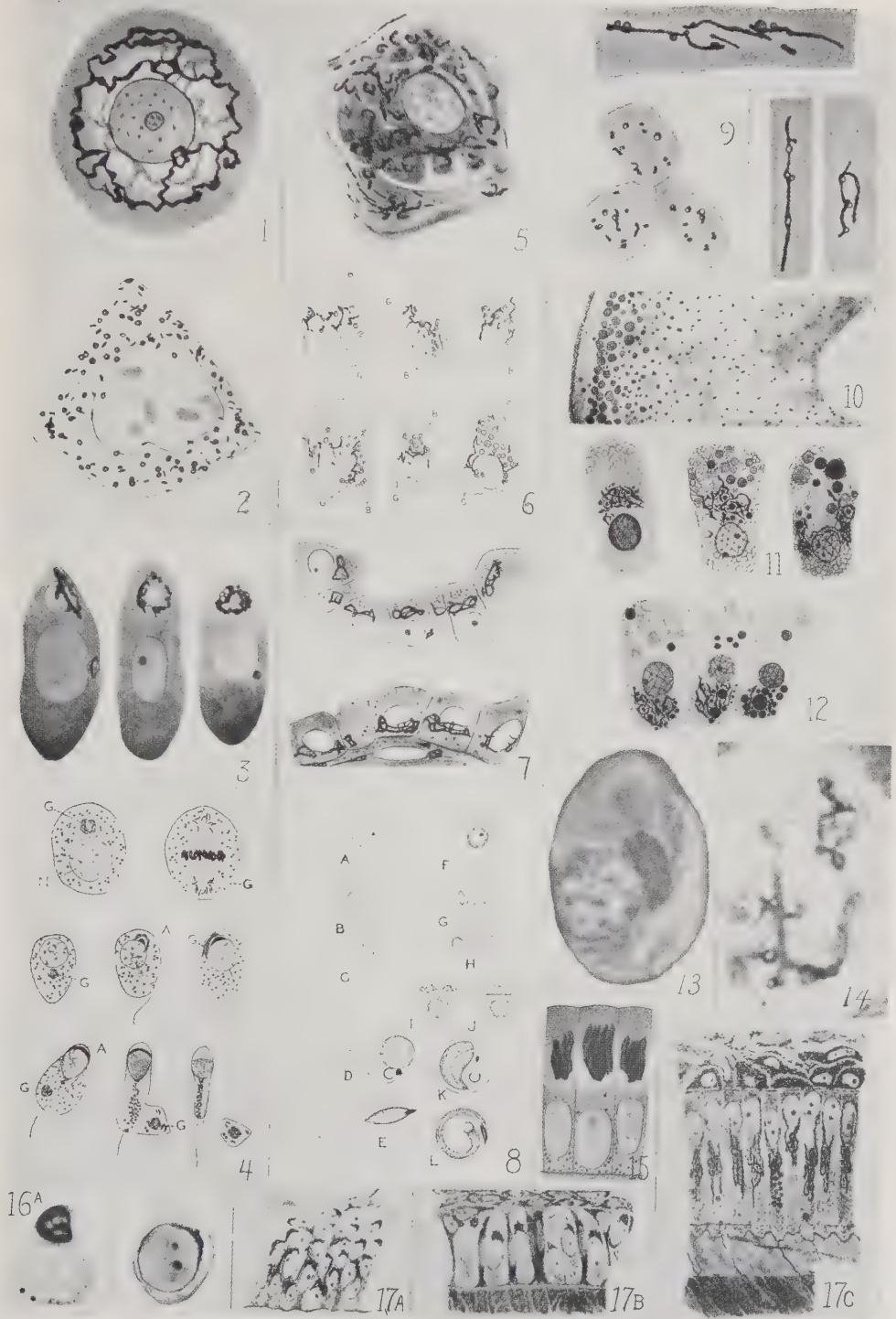
Comparison of spermatogenesis in insects with sporogenesis and spermiogenesis in Polytrichum commune. From Weier ('33)

GOLGI APPARATUS	PLASTID
	<i>Sporogenesis</i>
1. In primary spermatocytes. A number of scattered Golgi bodies, with an outer heavier staining horse shoe-shaped border partially surrounding an inner pellicle of some lightly staining substance (A).	1. In archesporial cells. A duplex plastid characterized by a horse shoe-shaped heavily stained plastonema which partially surrounds a lighter staining pellicle, the plastosome (F).
2. During the maturation division. Dictyosome bodies undergo fragmentation and are distributed in equal amounts to the spermatids (B).	2. During the reduction divisions. The duplex plastid, at least under the influence of the mitochondrial fixatives, fragments into a mass of small granules, which are to be distributed to the spores (G).
3. In the spermatid. The mass of fragments fuse into a single body presenting the same staining appearance as the numerous bodies present in the spermatocyte. This single body is the acroblast (C).	3. During the anaphase of the second division. The mass of fragments destined for a single spore gather into a single body which presents the same figure as did the duplex plastid of the archesporial cell (H).
	<i>Spermiogenesis—androcytes</i>
	With the formation of the androcyte the plastid may be observed as a delicate chromophilic network embedded in a lightly differentiated cytoplasm (I). This structure soon develops into the limosphere, a body quite similar to the duplex plastid of earlier stages (J).
4. The acrosome is budded off the acroblast (D).	4. The apical body is budded off the limosphere (K).
5. The acrosome passes to the anterior end of the elongating nucleus. Depending upon the species it may or may not elongate (E).	5. The apical body passes to the anterior end of the elongating sperm where it too elongates (L).
6. The remaining portion of the acroblast, the Golgi remnant, passes to the posterior end of the elongating sperm. It is eventually sloughed off (E).	6. The remaining portion of the limosphere, the limosphere remnant, passes to the posterior end of the sperm. It eventually disappears (L).

PLATE 1

EXPLANATION OF FIGURES

- 1 Golgi apparatus in spinal ganglion cell of a newborn cat. Golgi technique. Slightly modified after Golgi (1899), from Heidenhain ('11).
- 2 Osmiophilic platelets in apical cell from growing-point of *Equisetum arvense* root tip. Kolatchev technique. After Bowen ('28 a).
- 3 Excretion apparatus (Golgi apparatus) in an infusorian (*Dogielella sphaerii*). Kolatchev technique. After Nassonov ('25).
- 4 Semidiagrammatic stages in the development of a human spermatid. A, primary spermatocyte during late growth period; B, metaphase of first spermatocyte division; C, spermatid immediately after the conclusion of the second spermatocyte division; D to H, earlier stages in the transformation of the spermatocyte into a sperm. Slightly modified after Bowen, from Arey ('30). A, acrosome; G, Golgi apparatus plus idiozome; N, nucleus.
- 5 Golgi apparatus (blackened) and intracellular canaliculi (clear) in gastric parietal cell of rat. Mann-Kopsch technique. After Beams and King ('32 b).
- 6 Golgi apparatus (G) and trypan blue droplets (B) in cells of convoluted tubules of mouse kidney. Figures arranged as a progressive series to illustrate successive stages in deposition of the dye. Mann-Kopsch technique. After Ludford ('28).
- 7 Golgi apparatus in thyroid follicular cells of guinea pig. A, Golgi apparatus in usual position between nucleus and follicular lumen. Cajal technique. B, Golgi apparatus in reversed position between nucleus and interfollicular tissues. Da Fano technique. After Cowdry ('22), from Hertwig ('29).
- 8 Comparison of spermatogenesis in insects with sporogenesis and spermiogenesis in *Polytrichum commune*. For description see table 1. Slightly modified after Weier ('33).
- 9 Golgi apparatus with associated droplets (secretory granules?) from intestinal goblet and pancreatic cells. Kolatchev technique. After Bowen ('24 a).
- 10 Drawing showing distribution of Golgi elements and their relation to yolk spheres in *Cambarus oocyte*. Kopsch technique. After Kater ('28).
- 11 Entodermal cells of yolk sac in white mouse during secretion. The Golgi apparatus occupies a supranuclear position and holds smallest secretory droplets in its meshes. Kolatchev-Nassonov technique. After Litwer ('28).
- 12 Entodermal cells of yolk sac in white mouse during resorption of fat. The Golgi apparatus occupies a subnuclear position and holds the smallest fat droplets in its meshes. Kolatchev-Nassonov technique. After Litwer ('28).
- 13 Degranulated basophile cell from anterior hypophysis of guinea pig. The Golgi apparatus has been bleached, then restained with aniline blue. Kolatchev-Nassonov fixation and Heidenhain's azo carmine-aniline blue staining. Photomicrograph; Leitz 2 mm. apochromatic oil immersion objective and $\times 8$ periplan ocular.
- 14 Golgi apparatus in cervical spinal ganglion cell of guinea pig. Individual strands of the reticulum contain at irregular intervals small, round, vesicles; compare with figure 9. Kolatchev-Nassonov technique plus Heidenhain's azo carmine aniline blue staining. Photomicrograph; Leitz 2 mm. oil immersion objective and $\times 8$ periplan ocular.
- 15 Non-fenestrated, plate-like Golgi apparatus in prostatic cells from a 20-day castrate rat injected with testis extract. Kolatchev-Nassonov technique. After Moore, Price and Gallagher ('30).
- 16 Compact reticular type of Golgi apparatus in chromophobes of rat hypophysis. A, cell with basophile type of apparatus. B, cell with acidophile type of apparatus. Kolatchev technique. After Severinghaus ('33).
- 17 Golgi apparatus in cells of enamel organ. A, Golgi apparatus situated in the external ends of the inner epithelial cells at the point where the bending of the outer epithelium of the enamel organ and its transition into the inner one takes place; B, Golgi apparatus disposed in the outer ends of the young and less differentiated ameloblasts; C, Golgi apparatus, of ameloblasts, that has migrated to opposite ends of cells. Kolatchev-Nassonov technique. After Jasswoin ('25).



THE EFFECT OF MALE HORMONE SUBSTANCES UPON BIRTH AND PRENATAL DEVELOP- MENT IN THE RAT ¹

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Interference with the normal birth mechanism in rats results from excessive amounts of various hormones, such as from pituitary and placental extracts, and pregnancy urine (Teel, '26; Evans and Simpson, '29; Levin, Katzman and Doisy, '31; Hoopes, '34; Hain, '32), pituitary implants (Engle and Mermod, '28; Hain, '32), luteal extracts (Nelson, Pfiffner and Haterius, '30), hypophysectomy (Pencharz and Long, '33), and estrin (Parkes, '30; Hain, '35). Influence upon the processes of parturition is not limited to pituitary and female hormones, however, as shown by the data of the present paper concerning the effect of androgenic material upon parturition and prenatal development.

MATERIAL AND METHODS

Vaginal smears were made upon mated female rats for the detection of pregnancy (finding of sperm) and the animals separated and weighed daily during the duration of the pregnancy. At various intervals from 10 to 17 days after the finding of sperm in the vaginal smear, daily injection of testosterone propionate ² was begun. Subcutaneous injections of 500 gamma of testosterone propionate were continued daily until parturition or until autopsy of the animal. In an additional eight animals not listed in table 1, only one injection

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² Furnished through courtesy of Ciba Company under the trade name, Perandren.

was given. One-tenth cubic centimeter of peanut oil was used as a standard vehicle, since the type and quantity of oil influences the effectiveness of the hormone (Parkes, '36). Animals which failed to give birth were sacrificed 1 to 5 days subsequent to the day of expected parturition.

RESULTS

Pregnancy was considered to exist in only those rats which besides containing sperm also showed satisfactory daily increase in weight and either gave birth to young or were found

TABLE 1

Daily androgen injections (0.5 mg. testosterone propionate) prevent parturition if administration is begun the first to the fourteenth day, but not when started the sixteenth day or later. Under the experimental conditions employed, the fifteenth day is a crucial time, for when injections are begun at that time, parturition is prevented in approximately half of the animals

<i>Day of gestation injections begun</i>	<i>Number of rats</i>	<i>Number of rats giving birth</i>
10	2	0
11	2	0
12	2	0
13	6	1
14	4	0
15	7	3
16	4	3
17	3	3
	30	

at autopsy to show fetal sites. Thirty pregnant females were observed with regard to the occurrence of parturition (table 1) and to the description of the uterus, ovaries, and fetuses.

Effect on parturition. There was a distinct division between the earlier days of pregnancy in which injections of androgenic substance prevented development and birth, and the later days in which injections failed to prevent parturition. Of sixteen animals injected beginning the fourteenth day³ or earlier, all but one failed to give birth. Of seven animals not begun until the sixteenth or seventeenth day all but

³ Day no. 1 of the gestational period was considered to begin the day sperm were found in the vagina and to include the next 24 hours. Injections were initiated the last hour of the day stated in table 1.

one gave birth, although parturition was delayed in some instances. The fifteenth day was intermediate between these two periods, three of seven animals giving birth.

Fetuses. a. Size and viability. Fetuses from animals in which injections were begun earliest exhibited arrested development in many cases. Occasionally one to three large fetuses accompanied several smaller ones which were lacking in development. Even the larger ones were not always viable, but the lack of viability and the high percentage of those showing maceration may be due at least in part to the delay of autopsy 3 or more days after the expected day of parturition. Some remained viable, however, for several days after the expected parturition, and those which were born alive were generally successful in nursing and growing post-natally. With the prolongation of gestation beyond the normal date for parturition there was at most but slight increase in the size of the fetuses, which weighed 5 to 6 gm. as compared to 4 to 5 gm. for those born at term. None of the fetuses which remained in the uterus for 24 or 25 days showed the excessive size described for those carried post-term in the uterus by pregnancy urine extracts (Hoopes, '34).

b. Abnormal development resulting from androgenic administration. A most striking somatic difference between the young obtained from androgen-treated mothers as compared to those from uninjected mothers was the masculine appearance of all young, whether male or female, which were obtained from androgen-injected animals. Superficially almost all appeared somewhat like males, but operative inspection revealed that approximately one-half of the young were females, possessing ovaries, uterus and at least part of a vagina. The orifice of the vagina was sometimes absent, the proximal part being small but present. The clitoris was large and penile-like, and the distance between clitoris and rectum was sufficient to resemble the scrotal space seen in the male animal. Skene's ducts, which as the homologue of part of the male prostate have been shown to respond vigorously to androgen injections in adult females, were regularly developed. As has

been pointed out earlier, however, development of these ducts is common at the time of birth (Korenchevsky and Dennison, '36; Hamilton and Wolfe, '37). When these young grew to maturity the females which lacked the perineal orifice of the vagina, presented an imperforate vagina and extreme underdevelopment of the nipple, but the ovaries and uterus were of large size and grossly normal in appearance. Follicles, eggs and corpora were present. Daily abdominal inspection of the ovaries and uterus revealed cyclic changes. Sperm were found in the ejaculate of the males, when they had grown to maturity.

Reproductive organs of the androgen-injected pregnant rat

The uterus was characterized by thin, expanded cornua and a small, hard, firmly-contracted cervix. The cervix resisted strongly any attempt at dilation by insertion of an instrument in the os uterus. The fetuses were but seldom found to have engaged in the cervix; generally they were 1 cm. or more above the os uterus. The ovaries usually presented large corpora lutea. Skene's ducts were highly developed, often bilobed (Hamilton and Wolfe, '37). The preputial glands exhibited pronounced growth.

DISCUSSION

Androgens, like estrogens (Hain, '35 a), may result in delayed birth when injected in the latter days of gestation and failure of birth when injected in the earlier days (table 2). With the constant daily dose employed in the present experiments the fifteenth day is intermediate between the earlier period in which death results and the later period in which birth is not prevented (table 2). In many of the fetuses of injected mothers proper development was also prevented. So there may well be at least two factors at play, 1) a disruption of the birth mechanism and 2) an underdevelopment and abnormal development of the fetuses.

The question arises as to whether the androgens produce their effect by acting directly upon the uterus, indirectly through the endocrine system, or by both means. Testosterone

propionate can produce growth of the uterus in ovariectomized (Korenchevsky and Dennison, '35), hypophysectomized and hypophysectomized-ovariectomized rats (Leonard, Sager and Hamilton, '37). Histologically, uterine sections of androgen-treated puppies resemble in part the changes produced by luteal substances (Hamilton and Allen, '38). Robson ('37) and Leonard and co-workers ('37) have shown a luteal-like effect of androgenic material upon the rabbit uterus, but no statement is made as yet as to whether or not some of the effects reported in this paper are due to a luteal-like action. Noteworthy in this connection, however, are the findings

TABLE 2

Comparison of the effects of estrogenic and androgenic substances upon the birth mechanism. The data concerning estrogens is from work by Hain ('35) and applies to injections spaced over a period of 12 hours. The data concerning androgens is from this paper and applies to injections continued daily when begun

<i>Days of gestation when injections begun</i>			
Estrogen	1-15		16-22
ketohydroxyoestrin	Death or		Parturition not prevented
0.5-1.0 mg.	abortion		
Androgen	1-14	15	16-20
0.5 mg. daily	Death or	Half give birth;	Parturition not prevented
testosterone propionate	abortion	half prevented	but sometimes delayed

(Wolfe and Hamilton, '37 a) that androgens are capable of causing luteinization of the rat ovary and are much more effective in producing luteinization when administered at estrus rather than at diestrus. That androgens affect the pituitary has been demonstrated histologically (Nelson and Gallagher, '35; Wolfe and Hamilton, '37 a, b, c) and physiologically (Moore and Price, '37; Hamilton and Wolfe, '38).

It is possible that the hard, firm cervix found in the pregnant animal is responsible for the delay in birth. The cervix can be dilated only with difficulty as seen upon insertion of a pair of forceps, and it will resist expulsion of the fetuses by manual compression of the cornua. Nevertheless, this does not seem to be the whole answer because in many animals the

fetuses had not progressed to the region of the cervix but were only in the horns. It may be that the contractile response of the uterus is lessened in androgen-injected animals. Such a suggestion recalls the flabby type of uterus seen in the androgen-injected rat (Leonard, Sager and Hamilton, '37) and puppy (Hamilton and Allen, '38) as compared to the swollen, distended uterus seen in the estrogen-injected rat (Korenchevsky and Dennison, '35) and the luteal-like action of androgens upon uterine contractions (Robson, '37; Leonard, Sager and Hamilton, '37).

The masculine characteristics observed in the female young of androgen-injected mothers is further evidence of the masculinizing properties of androgenic substances on female animals. In testing possible usefulness of these substances in the female, as in the prevention of miscarriage, or in alleviation of menopausal symptoms, it should be remembered that they may affect the young and also possess a striking masculinizing effect on the female in producing growth changes in those organs whose homologues in the male animal respond to male hormonal substances (Hamilton, '37). This applies especially to the clitoris (Hamilton, '37) and Skene's ducts (Hamilton and Wolfe, '37).

It is possible that some degree of masculinizing action may perhaps be necessary for normal clitoridean and urethral growth. Injections of estrogens into pregnant rats produce a hypospadiac condition (Hain, '35 b; Greene, '37).

The facts that by the presence of androgenic material in the mother 1) abnormal development of secondary sexual organs can be accomplished prenatally in the young, 2) the clitoris and perineal parts can be transformed to assume a masculine, falsely hermaphroditic appearance, and 3) the development of some female organs of reproduction is prohibited, as for example the distal part of the vagina, are of significance in an understanding of the production of pseudohermaphroditism and abnormal prenatal development.

SUMMARY

Thirty pregnant rats were given daily injections of androgenic substance, 500 gamma of testosterone propionate in oil, starting the tenth to the seventeenth day of pregnancy. Parturition was delayed in those receiving the injections in the latter days of pregnancy, prevented and fetal development hindered in those receiving injections in the earlier days. Administration begun on the fifteenth day of gestation, which is intermediate between periods causing delay and prevention, resulted in delay in approximately half of the animals, prevention in the other half.

All young of androgen-injected mothers presented a masculine appearance externally. The females exhibited a large penis-like clitoris and scrotal-like perineum, and in some cases lack of development of female reproductive organs, especially the perineal orifice of the vagina. This is considered suggestive as to a possible cause of false hermaphroditism and embryological defects in the female. When the affected female young had grown to maturity the reproductive system was functional but the outer portion of the vagina remained undeveloped and imperforate and the nipples markedly undeveloped.

LITERATURE CITED

- ENGLE, E. T., AND C. MERMOD 1928 The action of the gonad-stimulating hormone of the anterior lobe on the course of pregnancy in the rat and mouse. *Anat. Rec.*, vol. 38, p. 11.
- EVANS, H. M., AND M. E. SIMPSON 1929 Impairment of the birth mechanism due to hormones from the anterior hypophysis. *Proc. Soc. Exp. Biol. and Med.*, vol. 26, pp. 595-597.
- GREENE, R. R. 1937 Production of experimental hypospadias in the female rat. *Proc. Soc. Exp. Biol. and Med.*, vol. 36, pp. 503-506.
- HAIN, A. M. 1932 The physiology of pregnancy in the rat. I. The prolongation and interruption of pregnancy. *Quart. J. Exp. Physiol.*, vol. 22, pp. 249-267.
- 1935 a The physiology of pregnancy in the rat: an hormonal investigation into the mechanism of parturition. Effect on the female rat of the ante-natal administration of oestrin to the mother. *Quart. J. Exp. Physiol.*, vol. 25, pp. 131-143.
- 1935 b An effect on the rat of antenatal and postnatal administration of oestrin. *Edinburgh Med. J.*, vol. 42, pp. 101-112.
- HAMILTON, J. B. 1937 Masculinizing effect of male hormone substance upon the female reproductive tract. *Anat. Rec.*, vol. 67 (supplement), p. 22.

- HAMILTON, J. B., AND E. ALLEN 1938 Unpublished data.
- HAMILTON, J. B., AND J. M. WOLFE 1937 Prostatic type of paraurethral glands induced in female rats by administration of male sex hormone. *Proc. Soc. Exp. Biol. and Med.*, vol. 36, pp. 465-468.
- 1938 The effect of synthetic androgen upon the gonadotropic potency of the anterior pituitary. *Endocrinology*. In press.
- HOOPES, E. C. 1934 Prolonged pregnancy in albino rat following injection of pregnancy urine extract. *Proc. Soc. Exp. Biol. and Med.*, vol. 31, pp. 1115-1117.
- KORENCHEVSKY, V., AND M. DENNISON 1936 The histology of sex organs of ovariectomized rats treated with male or female sex hormone alone or with both simultaneously. *J. Path. and Bact.*, vol. 42, pp. 91-104.
- 1936 The histological changes in the sex organs of spayed rats induced by testosterone and oestrone. *J. Path. and Bact.*, vol. 43, pp. 345-356.
- LEONARD S. L., V. SAGER AND J. B. HAMILTON 1937 Effect of male hormone upon uterine motility and the uterus. *Proc. Soc. Exp. Biol. and Med.*, vol. 37, pp. 362-365.
- LEVIN, L., P. S. KATZMAN AND E. A. DOISY 1931 Effects of estrogenic substances and the luteinizing factor on pregnancy in the albino rat. *Endocrinology*, vol. 15, pp. 207-213.
- MOORE, C. R., AND D. PRICE 1937 Some effects of synthetically prepared male hormone (androsterone) in the rat. *Endocrinology*, vol. 21, pp. 313-329.
- NELSON, W. O., AND T. F. GALLAGHER 1935 Studies on the anterior hypophysis; effect of male hormone preparations upon anterior hypophyses of gonadectomized male and female rats. *Anat. Rec.*, vol. 64, pp. 129-145.
- 1936 Some effects of androgenic substances in the rat. *Science*, vol. 84, pp. 230-232.
- NELSON, W. O., J. J. PFIFFNER AND H. O. HATERIUS 1930 The prolongation of pregnancy by extracts of corpus luteum. *Am. J. Physiol.*, vol. 91, pp. 690-695.
- PARKES, A. S. 1930 On the synergism between oestrin and oxytocin. *J. Physiol.*, vol. 69, pp. 463-472.
- 1936 Increasing the effectiveness of testosterone. *Lancet*, vol. 2, pp. 674-676.
- PENCHARZ, R. I., AND J. A. LONG 1933 Hypophysectomy in the pregnant rat. *Am. J. Anat.*, vol. 53, pp. 117-139.
- ROBSON, J. M. 1937 Reactions of the uterine muscle and endometrium of the rabbit to testosterone. *Quart. J. Exp. Physiol.*, vol. 26, pp. 355-359.
- TEEL, H. M. 1926 The effects of injecting anterior hypophysial fluid on the course of gestation in the rat. *Am. J. Physiol.*, vol. 79, pp. 170-183.
- WOLFE, J. M., AND J. B. HAMILTON 1937 a Action of male sex hormone with and without estrin in the female rat. *Proc. Soc. Exp. Biol. and Med.*, vol. 37, pp. 189-193.
- 1937 b Response of anterior pituitary of immature castrated rat to testosterone and related compounds. *Proc. Soc. Exp. Biol. and Med.*, vol. 36, pp. 307-310.
- 1937 c Comparative action of testosterone compounds, of estrone and of combinations of testosterone compounds and estrone on the anterior hypophysis. *Endocrinology*, vol. 21, pp. 603-610.

STUDIES ON CARDIAC MUSCLE CELLS, FROM CHICK EMBRYOS, GROWN IN TISSUE CULTURE

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ONE FIGURE

One of the authors of the present paper (Hogue, '37) has recently grown embryonic cardiac muscle tissue in a specially prepared liquid culture medium. With the method used cardiac muscle cells were kept alive for 180 days. The most important result of this work in cultivating embryonic cardiac cells was the fact that in the special medium the migrating cells could be maintained at a comparatively high degree of differentiation. In cultures 30 to 40 days old, the majority of the migrating cardiac cells showed distinct fibrillae, often with optical segmentation. Only occasionally were cells observed with no trace of a fibrillar structure.

It is clear from Hogue's description that not all the fibrillated cells were in the same stage of differentiation. Sometimes a large number of fibrillae appeared, occupying the entire body of the cell. In such cases the distinctness of the individual fibrillae was somewhat obscured. In other cells only a small bundle of fibrillae was found embedded in a comparatively large amount of unfibrillated sarcoplasm. In exceptional cases a solitary fibrilla appeared in the otherwise undifferentiated sarcoplasm. In both of these types of partially differentiated cells the myofibrillae, and frequently their optical segmentation, was as distinct as that seen in the fixed and stained preparations of the same set of cultures.

Cells cultivated in the medium prepared by Hogue offered a favorable subject for studying 1) the nature and 2) the functional properties of the fibrillar inclusions of cardiac muscle cells grown from small pieces of chick embryo heart.

Every cytologist is familiar with the literature concerning our first problem, i.e., the nature of fibrillar inclusions. It is well known that hundreds of attempts have been made to ascertain whether the fibrillar inclusions, especially those appearing in the muscle and in the nerve cells when treated with fixatives, are only products of technique or whether they are preexistent and become more distinct by the application of chemicals. In order to obtain more information on this particular subject the following experiments were made with living cardiac cells in tissue culture.

Cultures were transferred to the moist chamber of a microdissecting apparatus. Individual cells, with only a small bundle of fibrillae in the undifferentiated sarcoplasm were studied with high power lenses. The number and distribution of the fibrillae as well as the position of the granules of the unfibrillated sarcoplasm were carefully recorded. Sometimes the continuous and vigorous, rhythmic contraction of the cells interfered with accurate observation. To overcome this difficulty the cells were brought to rest by lowering the temperature of the cultures to 34 to 35°C. In this resting condition, the most minute details of the interior of the cells could be successfully studied. This slight cooling of the cultures for about 30 minutes had no noticeable effect upon the vitality of the cells. As soon as the temperature was restored to normal, rhythmic contraction began again and the cells continued to live for many weeks.

After the cells had been studied while resting but still alive, they were treated with a series of chemicals used in histological laboratories as routine fixatives (10% formaldehyde, saturated solution of mercuric chloride, 95% alcohol; 1 to 10% solution of silver nitrate; saturated solution of picric acid, 2% solution of osmic acid; 3 to 5% solution of acetic acid, etc.). All these solutions were injected with the aid of

micropipettes into the liquid culture medium and their effect upon the cells was observed with high power lenses. Some of the cells responded to the chemicals with a vigorous contraction. The majority of them, however, were fixed at once. Our attention was concentrated mostly on those changes which occurred in the interior of the cells upon the application of chemicals. The following are our observations. The transparent, hyaline matrix of the cells became slightly opaque when treated with the above chemicals. Individual fibrillae were brought closer together, so that the bundle of fibrillae was found to be much narrower after fixation than before. The individual fibrillae, however, did not seem to change their structure during fixation. In contrast to this the granular inclusions of the cells suffered serious damage. Some of the granules dissolved completely; others were broken up into smaller fragments and the rest seemed to be unaffected. However, none of the chemicals applied to the tissue culture cells displaced the granules. We could not find the slightest evidence to support the theory that, under the influence of fixatives, granular inclusions rearrange themselves into lines or rows. Neither did any of the chemicals produce threads or fibrillae in that part of the cell which appeared to be a structureless matrix before the treatment.

From these experiments it is clear that the effect of fixatives was restricted to the destruction of certain granules in the cell and that the fixatives preserved all those myofibrillae which existed previous to treatment, but never caused the formation of additional fibrillae.

In another series of experiments we studied the effect of stress upon the partially fibrillated cardiac cells in the following way. Resting cells were pulled with a microneedle at right angles to the direction of their fibrillae. Some of the cells contracted at the touch of the microneedle; others remained in the resting condition. The fibrillae, following the pull, deviated from their original position. As a consequence the bundle of fibrillae now appeared to be more loosely arranged than before. The granules in the unfibrillated part of

the cell were also affected by the pull. Some were seen to move in the direction of the pull, others to move in the direction opposite to the pull. A third group of granules moved rapidly in various directions until they settled down in their final position in the stretched cell. In no case did they show a tendency to rearrange themselves in rows, or lines, following the direction of stress. The optically homogeneous sarcoplasm was not affected by the pull. No stress lines or striations appeared in this part of the cell during the experiment, which lasted from 5 to 8 minutes.

When the cell was released from the pull the granules shifted again. Some moved back toward their original place, others remained in their displaced position. Certainly the majority were far from their original position. This shifting of the visible granules indicated to us that the position of the invisible particles was also disturbed by the stretching and releasing of the cell. However, this severe disturbance in the interior of the cells did not interfere with their ability to contract, for we found that cells after being pulled for 5 to 8 minutes and then released would contract normally when the temperature was again raised to 37 to 38°C. Results similar to the above were obtained when cells were stretched in a direction parallel to that of their fibrillae.

These experiments showed that stress, if applied for a short period of time, has a very limited effect upon the cells. Stress may force the formed elements of the cell to change their positions but no new structures are formed under its influence. Stretching the cells for a longer period of time (10 to 15 minutes) causes them to disintegrate.

In another series of experiments cultures were used in which the cells were contracting rhythmically. Here we concentrated our attention particularly on that problem which has been so much discussed in the last decade by histologists and physiologists. It can be resolved into three questions: 1) should myofibrillae be considered as the only structures which are endowed with contractile properties in the muscle cells; 2) is the unfibrillated sarcoplasm alone responsible for

the contractility of muscle tissue; 3) are they both contractile? Evidence on these points should be obtained by studying cells possessing a bundle of myofibrillae on one side and unfibrillated sarcoplasm on the other. If it be true that the myofibrillae are the only contractile components, one would expect that during contraction the fibrillated portion of the cell would become shorter than the unfibrillated portion, and that the shape of the unfibrillated part would be considerably distorted. If, on the other hand, the sarcoplasm is the only contractile substance, one would expect that the myofibrillae would assume a wavy course in contracted cells.

We studied contraction in both fibrillated and non-fibrillated cardiac muscle cells. The temperature was kept at 37°C. No chemical or mechanical stimuli were used. During contraction neither part of the cell was found to act independently. The unfibrillated portion became shorter and stouter. The fibrillae after contracting were still straight, instead of appearing as wavy lines, which one would expect of non-contractile fibrillae embedded in a contractile matrix.

From our experiments we are convinced that the cells with fibrillae contracted in the same way as did those without fibrillae. Obviously, if the structural differentiation which led to the appearance of fibrillae had any influence upon the contractile activities of the cells this influence was of such a minor nature that it could not be detected with the microscope.

Thus we have found, that, even in the early embryonic state, the myofibrillae must be considered as a highly specialized form of contractile substance. Tissue culture material offers no evidence for the theory that myofibrillae, when once formed, are the only contractile elements. The sarcoplasm continues its contractile function undisturbed by the presence of myofibrillae.

Since previous investigators working with living embryonic cells have stated that sarcoplasm might contract without the aid of myofibrillae, we were curious to know whether the reverse condition could be established by experimental means; i.e., could myofibrillae be made to contract independently of

the sarcoplasm? This problem required a separation of myofibrillae from the sarcoplasm. After sacrificing a large number of cells and after repeated failures, we devised two methods by which we were able to do this.

1. The micropipette was filled with distilled water and its tip brought close to an active muscle cell on the cover slip. The distilled water was then injected into the culture medium. There was considerable variation in these experiments, for the cells were not all in the same stage of development, and the culture medium could not be diluted uniformly because the amount of injected liquid could not be accurately controlled. As a result of these differences the cells used in these experiments showed a considerable degree of variation in their response to the stimulus. However, in one respect the cells all behaved alike. Cells which had been contracting normally before the injection, split into two parts as soon as the culture medium was diluted. The unfibrillated portion stopped contracting at once, absorbed water and became swollen (fig. 1). Its granules were seriously injured. Some of them were dissolved, others showed brownian movement.

The fibrillae at first were not so seriously affected. They continued to contract for longer or shorter periods of time. In some cases they would twitch only two or three times, in others a long series of contractions occurred within a sarcoplasm that was completely paralyzed and whose granules were dissolved. The myofibrillae broke up into granules shortly after they stopped contracting.

Since we have found in previous studies that there is no regularity in the beat of cardiac muscle cells in tissue cultures we made no attempt to study the rate of contraction of the myofibrillae when exposed to distilled water. However, the contractions of the myofibrillae within the disintegrated sarcoplasm were not identical with those of the normal cell. On an average, contraction of the myofibrillae was less frequent and more irregular. Thus while the length of the intervals between the contractions was gradually increasing, at the same time incomplete twitches were occurring between the strong

contractions. In one of our most successful experiments contraction of the myofibrillae was observed 12 minutes after the sarcoplasm had been paralyzed with distilled water.

2. The second method of separating the fibrillated from the unfibrillated portions of the cardiac muscle cells was as follows: with the aid of the microneedle a slight break was made in the surface membrane. This was sufficient to effect the separation. The sarcoplasm absorbed liquid through the opening

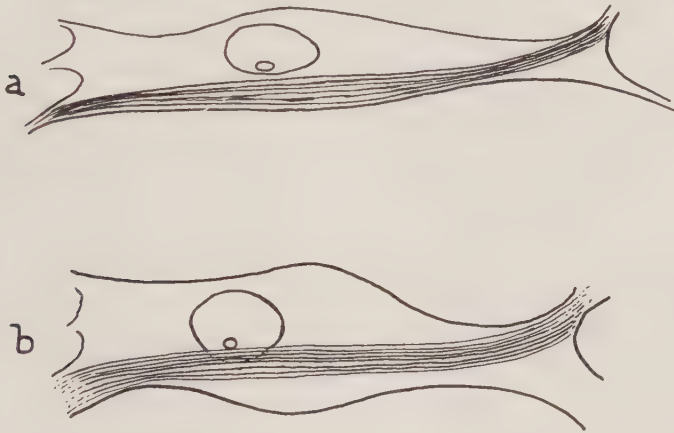


Fig.1 Camera lucida drawings of a cardiac muscle cell grown in tissue culture: a, in the resting state in tissue culture medium. b, in the resting state in diluted tissue culture medium. The myofibrillae of this cell contracted 12 minutes after the culture medium was diluted, whereas the sarcoplasm was paralyzed when the distilled water was added to the medium.

and became paralyzed while the fibrillae behaved in the same manner as did those which had been treated by the first method.

DISCUSSION

It is well known that certain histological procedures may cause serious damage to cells. Chemicals may injure the cells and produce a granular framework in parts which were previously transparent; they may also dissolve preexisting components of the cell—such as granules and mitochondria—or at least break them up into smaller pieces. Artifacts such

as these are well known to cytologists working with living cells. However, we cannot classify myofibrillae as artifacts for they actually exist in the living embryonic cardiac muscle cell as distinct fibrillae. They are undoubtedly structures of the cells themselves and are not produced by the technique.

As regards the contractile nature of the different components of muscle cells the reader is referred to the publications of Lewis and Lewis ('15, '17, '20, '24), Bozler ('28), Friedheim ('31), de Rényi and Hogue ('31, '34).

Lewis and Lewis first established the fact that sarcoplasm is a contractile substance. These authors have also shown that under certain conditions the undifferentiated sarcoplasm may perform various types of contractions without the aid of a myofibrillar structure. Bozler overlooked the results of Lewis and Lewis and supported the theory that sarcoplasm does not have the power to contract. According to him various types of myofibrillae develop within the muscle cell, each fibrilla having its own definite type of contraction. Friedheim assumed that no contraction occurs until optical segmentation develops in the cell. Our experiments confirm the observations of Lewis and Lewis. In addition we succeeded in bringing the undifferentiated sarcoplasm to a tonic type of contraction by the application of a mechanical stimulus.

From the results of our previous and present studies we conclude that the organization necessary for the various types of contractions is already present in the embryonic, undifferentiated sarcoplasm. The contractile activities of the sarcoplasm are not suppressed by the presence of myofibrillae. Both the sarcoplasm and the myofibrillae are forms of contractile substance which, in the embryonic state and under normal conditions, act synchronously. Independent action of one of the contractile components occurs only when the muscle cell is exposed to artificially changed conditions. Therefore the independent response of either of the contractile components to strange stimuli should not be considered as evidence for its dominating role in the process of contraction.

SUMMARY

Muscle cells from chick embryos were cultivated in a special liquid medium. The migrating cells contained small bundles of myofibrillae in the granular sarcoplasm. The cells were exposed to chemical solutions and to mechanical stress. The effect of these upon the myofibrillae and granular sarcoplasm was observed with high power lens. No support could be found for the theory that fibrillar structures in muscle cells are artifacts. Both myofibrillae and sarcoplasm were found to be forms of contractile substance, acting synchronously under normal conditions. When the sarcoplasm was paralyzed the myofibrillae were still active for longer or shorter periods of time.

LITERATURE CITED

- BOZLER, E. 1928 Weitere Untersuchungen zur Frage des Tonussubstrates. *Z. vergl. Physiol.*, Bd. 8, S. 371.
- FRIEDHEIM, E. A. H. 1931 Morphologische und funktionelle Untersuchungen an isolierten, in vitro gezüchteten Skelettmuskelfasern. *Arch. exp. Zellforsch.*, Bd. 11, S. 385.
- HOGUE, M. J. 1937 Studies of heart muscle in tissue cultures. *Anat. Rec.*, vol. 67, p. 521.
- LEWIS, M. R. 1915 Rhythmical contraction of the skeletal muscle tissue observed in tissue cultures. *Am. J. Physiol.*, vol. 38, p. 153.
- 1920 Muscular contraction in tissue cultures. *Contrib. to Embryol.* no. 35 (Carnegie Insti.), vol. 9, p. 191.
- LEWIS, W. H., AND M. R. LEWIS 1917 Behavior of cross striated muscle tissue cultures. *Am. J. Anat.*, vol. 22, p. 169.
- 1924 Behaviour of cells in tissue culture. *General Cytology*. Edited by E. V. Cowdry, Chicago, Univ. Press, p. 385.
- DE RÉNYI, G. S., AND M. J. HOGUE 1931 Studies made by means of microdissection on skeletal muscle grown in tissue culture. *Arch. exp. Zellforsch.*, Bd. 11, S. 304.
- 1934 Studies on skeletal muscle grown in tissue cultures. *Arch. exp. Zellforsch.*, Bd. 16, S. 167.

THE TOPOGRAPHY OF THE HYPOPHYSIS IN THE XENARTHRA

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FOUR PLATES (TWELVE FIGURES)

The present study gives an account of the topography of the pituitary gland in representatives of the three families of the Xenarthra. The hypophysis in these three groups of mammals is in some respects so unusual that it deserves to be described.

MATERIAL AND METHODS

The material comprises the adult pituitary glands from three two-toed sloths (*Choloepus hoffmanni*), three three-toed sloths (*Bradypus griseus griseus*), one anteater (*Tamandua tetradactyla*), and three armadillos (*Dasypus novemcinctus*). All of these animals were collected in the Republic of Panama, excepting the armadillos which were obtained from Texas.¹

The material was prepared for study by methods designed primarily to give information regarding the topography of the various parts of the gland, as well as to show their relationship to the brain. For this purpose the brain attached to the base of the skull was fixed in 10% formalin. The specimens were decalcified in 4% trichloroacetic acid, next embedded in celloidin and then cut serially in 20 μ sections in the sagittal plane. The sections were stained with haematoxylin and

¹ This investigation is incidental to work undertaken in tropical America on the reproduction of monkeys, under a grant from the Committee on Sex of the National Research Council. For aid in obtaining the material, the writer is greatly indebted to Mr. J. B. Shropshire. My sincere thanks are due, as well, to Dr. James Zetek and Dr. Herbert Clark who placed the facilities of their respective institutes, namely, the Barro Colorado Island Laboratory and the Gorgas Memorial Institute, at my disposal.

eosin. The material so obtained shows the pituitary gland lying in situ within the shallow sella turcica and attached by the infundibulum to the base of the brain.

OBSERVATIONS

Two-toed sloth (Choloepus hoffmanni). Figures 1 to 3. It will be noted that the pituitary gland of the two-toed sloth lies in a relatively shallow depression in the sphenoid bone. The gland is markedly flattened and consists of a well-defined, dark staining anterior lobe and a less deeply stained neural lobe.

The neural lobe is a long drawn out structure consisting of a lengthy stalk and a large but somewhat flattened infundibular process. An infundibular recess from the third ventricle extends throughout the length of the stalk into the infundibular process.

The epithelial portion of the gland is differentiated into a large pars distalis and a small pars intermedia, but shows no signs of the presence of a pars tuberalis. Moreover, the epithelial hypophysis does not enfold the neural lobe, but is attached only to a relatively small portion of the ventral surface of the stalk and to the antero-ventral aspect of the infundibular process. The neural stalk and process are lodged in a shallow groove upon the dorsal surface of the buccal pituitary. The pars intermedia, although not readily visible in the photographs, consists of a narrow strip of uniformly blue-staining cells bordering the infundibular process. A small residual lumen may or may not be present. In the specimen shown in figures 1 and 2 no residual lumen is visible, but in another specimen (fig. 3) a small crescentic space is seen separating a narrow strip of intermedia from the pars distalis. In the first specimen, which lacks a residual cleft, the pars intermedia is not sharply set off from the distal portion of the gland. The line of demarcation between the neural lobe and the pars intermedia, on the contrary, is exceedingly sharp and even (figs. 1 and 2), appearing almost as though a narrow, but distinct, capsule of connective tissue separated the two. This interpretation is supported by the find-

ing of scattered melanophores at the line of junction of the two tissues. In figure 3, of a specimen in which the blood vessels have been injected with India ink, the injected vessels obscure, more or less, the sharp contour of the line of contact of the pars intermedia and the neural tissue. Moreover, the dark strands extending upward from the pars distalis into the ventral part of the infundibular stalk, which might readily be taken in the photograph for epithelial strands, are in reality composed solely of blood vessels which, in this specimen, were filled with India ink by vascular injection.

A pars tuberalis appears to be completely lacking, the epithelial pituitary lying wholly within the sella beneath the sellar diaphragm. There is no evidence whatsoever of the presence of epithelial cells extending upward along the infundibular stalk to constitute a pars tuberalis as described in other mammals. Instead of a mantle of epithelial cells investing the infundibulum and the tuber cinereum, these regions are covered solely by a sheet of highly vascular leptomeninges. The blackish tongue of tissue (lm) in figure 1, situated on the surface of the ventrorostral aspect of the infundibular stalk which might be assumed at this low magnification to consist of pars tuberalis, is composed solely of leptomeninges which stain darkly because of the presence of numerous blood vessels as well as melanophores.

A further characteristic of the hypophysis of the two-toed sloth is the relatively heavy sheath of dura by which it is encapsulated. The dura consists of a mural portion lining the sella and of a diaphragmatic portion which forms an extremely heavy shelf overlying the entire infundibular process. The free edge of this shelf borders the infundibular stalk posteriorly. A lesser and more delicate dural fold adheres tightly to the dorsal surface of the anterior lobe and forms the anterior margin of the dural aperture transmitting the infundibular stalk. Moreover, the dura is adherent to the neural lobe as well as to the pars anterior, forming a capsule for the gland.

The dura, in two out of three specimens, is rather heavily pigmented due to the presence of melanophores. These are especially abundant in the dura at the rostral tip of the anterior lobe, and in the anterior dural lip of the diaphragmatic aperture. Melanophores are also present in the pia-arachnoid which surrounds the infundibular stalk, occurring especially in the adventitial sheaths of the larger pial vessels. Melanophores also penetrate the substance of the gland in places. A few are also encountered between the infundibular process and the pars intermedia, as well as in the neural stalk and in the tissue of the pars distalis.

Three-toed sloth (Bradypus griseus griseus). Figures 4 to 7. The hypophysis of the three-toed sloth is an extremely flattened structure lying in a slight hollow in the sphenoid bone. The neural lobe is similar to, but even more elongated than, that of the two-toed sloth. It possesses an extremely long, slender stalk, containing a commodious infundibular recess which extends into the flattened infundibular process.

In contact with the entire ventral surface of the neural stalk and its process lies the exceedingly flattened buccal portion of the gland. The latter consists of an anterior lobe separated from the neural portion of the gland by a thin sheet of pars intermedia. The pars intermedia is partially set off from the anterior lobe by several isolated, slender clefts which represent the residual lumen. The pars intermedia differs from that of the two-toed sloth by the fact that it extends far backward, forming a thin mantle adherent to the convex ventral surface of the infundibular process. At the extreme caudal tip of the neural lobe the pars intermedia exhibits a local enlargement. In the region of the middle portion of the neural stalk, the pars intermedia forms small flanges of tissue which embrace the sides of the stalk. Compared with the two-toed sloth, the boundary between the neural lobe and the pars intermedia is not distinct. This is due to penetration, to a slight degree, of the cells of the pars intermedia into the substance of the neural lobe, and the apparent absence of any special condensation of connective tissue between them.

The pars distalis consists of a thickened anterior mass lying ventral to the infundibular stalk, and of a thin shell-like portion which extends backward and is nearly co-extensive with the intermedia, excepting the above-mentioned caudal enlargement of the intermedia which is free. In the region of the middle third of the length of the horizontal infundibular stalk, both the pars intermedia and the pars distalis curve upward on the sides of the stalk, forming slight lateral cushions or flanges. Consequently the dorsal surface of the buccal hypophysis assumes the form of a shallow excavation in which the neural lobe rests.

The hypophysis of the three-toed sloth, similar to that of the two-toed sloth, is embedded in a stout dural capsule. The dura splits at the posterior pole of the pituitary into a mural sheet which lines the sella and into a heavy diaphragmatic sheet which adheres to the upper surface of the neural lobe. The latter extends forward for an extraordinarily long distance to terminate as the posterior border of the diaphragmatic aperture. At the rostral pole of the pituitary, where the anterior lobe extends forward like the bow of a boat, the dura cleaves similarly into dorsal and ventral components which encapsulate the gland. Melanophores are not observed in the hypophyseal meninges in the three-toed sloth.

Anteater (Tamandua tetradactyla). Figures 8 to 10. The hypophysis of the anteater is in general shape much like that of the two-toed sloth although in volume it is approximately twice as large. It lies in a long trough-like excavation of the sphenoid bone. The neural lobe is much elongated and the stalk is in the main solid, although a narrow infundibular recess with small diverticula, creating a crenellated appearance, extends approximately one-third of its length. In addition to this irregular passage communicating with the third ventricle, the stalk and infundibular process contain a number of small cavities situated more distally, some of which may communicate with the infundibular recess but the majority of which appear to be closed spaces. These minute cavities are lined by distinct ependymal cells. They contain abundant

secretion which stains deeply with eosin, besides numerous free cells which are basophilic. In the photographs the largest of these small cavities are at the limit of visibility.

Each lateral half of the anterior lobe gives rise to a mass of cells which encircles the neural stalk to fuse with a similar projection from the opposite side. In consequence the neural stalk just anterior to the expanded infundibular process is encircled by a conspicuous bridge composed of epithelial tissue. The main mass of this pontine structure consists of cells identical with the elements of the pars distalis. It possesses, however, a thin inner shell or covering of pars intermedia which separates the outer circumferential mass of granular cells from the neural stalk.

The configuration of the pars intermedia and of the residual lumen of the epithelial hypophysis is peculiar in the anteater. Besides being present in relationship to this bridge, the intermedia extends backward as a thin lamina lying between the anterior lobe and the ventral surface of the neural process, to the extreme caudal tip of the infundibular process, where it forms a thickened mass of cells, similar to that described in this region in the three-toed sloth. More extensive even than in the three-toed sloth, the intermedia can be traced around the sides and over the dorsal surface of the neural process. There is only a relatively small area upon the dorsal surface of the infundibular process that is not covered by a thin mantle of pars intermedia. In consequence of these peculiarities the pars intermedia of the anteater is extremely extensive and complex. Furthermore, the rostral part of the pars intermedia, associated with the above-mentioned bridge of epithelial tissue, differs in character from the part which covers the infundibular process. In the former locality the cells of the intermedia tend to be arranged to a considerable extent in minute follicles reminiscent of the histological appearance of pars tuberalis, whereas in the latter regions the cells are uniformly dispersed. A discussion of whether the rostral portion of the pars intermedia in the anteater should be homologized with the pars tuberalis of other mammals will be

presented in a later paragraph. Certainly the region in question does not coincide topographically with the location of the pars tuberalis in other mammals. The border line between the pars intermedia and the neural lobe is irregular, due to some infiltration of the neural tissue by epithelial cells of the intermedia.

As in the two sloths, no evidence can be found of the presence of tuberal prolongations of the buccal hypophysis along the neural stalk toward the base of the brain. The dark tissue (lm) seen in this region in figure 8 is composed solely of richly vascularized leptomeninges which contain many melanophores. The epithelial hypophysis lies entirely beneath the dural diaphragm spanning the sella. The dura, intimately fused with the surface of the hypophysis, is exceedingly thick and shows a topography similar to that encountered in the sloths. The dura throughout the basal region of the brain, but especially the sellar diaphragm, is quite deeply pigmented due to the presence of melanophores. The larger vessels traversing the leptomeninges are accompanied by numerous pigmented cells.

Armadillo (Dasypus novemcinctus). Figures 11 and 12. The hypophysis of the armadillo differs markedly in structure from that of either sloths or anteaters. It lies embedded in dura in an exceedingly shallow sella turcica. In mid-sagittal view it is seen to be somewhat ovoid in shape and not especially flattened. In contrast to sloths and anteaters the anterior lobe and the neural lobe are about equal in size. The neural stalk is relatively short and possesses no central lumen. The pars distalis of the epithelial hypophysis is attached to the antero-ventral surface of the neural lobe without any discernible intervening pars intermedia or residual lumen. The absence of pars intermedia is exactly the reverse of sloths and anteaters in which this portion of the gland is extremely well developed. Moreover, the pars distalis is sharply demarcated from the neural lobe, where the surfaces of the two are in apposition, indicating that no infiltration of the infundibular process by epithelial cells has taken place. Indeed, spindle-shaped cells, of the character of fibroblasts, appear to form a

delicate but continuous sheet intervening between the anterior and neural lobes. The dorsal surface of the anterior lobe exhibits a shallow excavation in which part of the ventral surface of the neural lobe rests. Near the junction of the infundibular stalk and process the anterior lobe creates a low flange of tissue on either side of the infundibular stalk. This is reminiscent of the complete bridge of epithelium enclosing the stalk in this region in the anteater and of the flange-like structures observed in the three-toed sloth. In complete contrast to the preceding animals, the armadillo possesses an extensive pars tuberalis which forms a mantle surrounding the infundibulum and extends well onto the surface of the tuber cinereum. On the anterior surface of the neural stalk especially, a sheet of epithelial cells extends rostrally to end in a conspicuous, wedge-shaped mass of cells attached to the tuber cinereum. Posteriorly the stalk is partially covered by the pars tuberalis, noticeable particularly as a wedge of epithelial cells in the angle between the infundibulum and the membranous floor of the ventricle contiguous to the mammillary body. Histologically the elements of the pars tuberalis are arranged in minute follicles. The dura is not quite so thick in the armadillo as in the sloths and anteaters. It encapsulates the body of the gland, however, as completely as in the previous animals. Melanophores are not visible in the meningeal coverings of the specimens of the armadillo.

DISCUSSION

The denial of the presence of a pars tuberalis in sloths and anteaters is based chiefly upon the failure to find a sheet or mantle of epithelial tissue which extends upward from the body of the pituitary, through the aperture in the sellar diaphragm, to surround the infundibulum and to spread out upon the surface of the tuber cinereum. Applying topographical criteria in the search for this portion of the gland, a pars tuberalis is certainly absent. The meaning of the term 'tuberal' part of the hypophysis obviously implies a portion of the epithelial tissue which is related to the tuber cinereum.

In all other mammals of which accounts exist, including various species of Marsupialia, Insectivora, Ungulata, Cetacea, Carnivora, Rodentia, Chiroptera, and Simiae, such an extension upward, compatible with the sense of the term 'tuberal,' has invariably been found. Heretofore, the absence of a pars tuberalis has been recorded only in certain lower vertebrates (Cameron, '29, fishes, some snakes and lizards).

It is possible, however, that cells equivalent to pars tuberalis might conceivably be found elsewhere in the pituitary complex of sloths and anteaters than in the customary location in other mammals. The diagnosis in this case would have to be made solely upon histological grounds without reference to topography. Adopting a purely histological criterion, the sloths present no signs of the epithelial pituitary being divided other than into a pars distalis and a pars intermedia, confirming our conclusion, on this ground as well, that the sloths do not possess a pars tuberalis.

In the anteater, on the contrary, the rostral part of the pars intermedia, which separates the conspicuous bridge of pars distalis from the enclosed infundibular stalk, is histologically different from the pars intermedia surrounding the infundibular process. This rostral portion between pars distalis and the neural lobe displays numerous minute follicles composed of cells which stain uniformly with haematoxylin and contain a droplet of eosin-stained colloid in the centers. Now the presence of such small follicles is one of the more constant histological features of the pars tuberalis of other mammals, so that it is probably valid to homologize this region in the anteater with the pars tuberalis of other mammals—accepting the histological evidence, although the topographical criteria do not coincide. In the sloths, on the contrary, in which this bridge of tissue is wholly lacking (two-toed sloth), or represented merely by two low flanges bordering the neural lobe (three-toed sloth), histological evidence of any epithelial tissue that can be homologized with the pars tuberalis appears to be totally wanting.

The fact that the pars tuberalis is lacking in both of the sloths points to their close kinship. Moreover, although present in the anteater, the pars tuberalis is distinctive in its topography from all other mammals which have been investigated. Finally, in the armadillo the pars tuberalis is present and well developed, possessing the customary topography of other mammals.

Interesting regarding the armadillo is the absence, on the other hand, of a pars intermedia, whereas the pars tuberalis is well represented. The fact that the armadillo's pituitary has no pars intermedia was already known to Mrs. M. R. Lewis, although the observation has never been published. I have a photograph of a dissection of the entire hypophysis of the armadillo, given to me by Mrs. Lewis, indicating quite clearly that there is no pars intermedia between the anterior and posterior lobes.

Other vertebrates in which a pars intermedia is lacking are known. In birds the pars intermedia is described as being reduced or absent, while in one group of mammals, the Cetacea, it is totally lacking (Wislocki, '29; Wislocki and Geiling, '36; Valsö, '34). Nevertheless, the whale's pituitary possesses other striking features, such as complete separation of the neural lobe from the pars anterior by the subdural space and a heavy fold of dura, which distinguish it from the hypophysis of the armadillo. The latter resembles more nearly in its topography the arrangement in birds in which the anterior lobe and neural lobes are in contact with one another, being separated at most by a delicate intervening film of spindle-shaped connective tissue cells.

These observations on the absence of a pars tuberalis in sloths, and on the lack of a pars intermedia in the armadillo and whale, indicate how variable the structure of the mammalian pituitary may be. The only constant portion of the pituitary appears to be the pars distalis. Physiologically this suggests that the main functions of the epithelial hypophysis reside in the anterior lobe and that whatever functions are assumed as being controlled by either pars inter-

media or pars tuberalis are either unessential in certain animals or are taken over by other parts of the organ which substitute for the absent part.

It is worth pointing out that in sloths and anteaters, in which the mantle of pars tuberalis surrounding the infundibulum is lacking, the rich blood supply, which is characteristic of the pars tuberalis in other mammals, is maintained in the form of an equally rich vascular plexus located in the sheath of leptomeninges surrounding the infundibulum. Thus the blood supply reaching the pituitary gland via the stalk appears to be independent of the presence or absence of the pars tuberalis.

The embryology of the pituitary of neither the Xenarthra nor the Cetacea being known, it would be unjustifiable to assume that neither the pars tuberalis nor the pars intermedia had ever developed. It is quite possible that they are formed initially in the early growth period, but that one or the other, as the case may be, undergoes subsequent reduction.

Finally we wish to call attention in the Xenarthra to the relations of the dura to the hypophysis. Compared to the majority of mammals—cat, dog, rhesus monkey, or man, for example—the dura investing the pituitary gland is exceedingly heavy and thick. This circumstance makes it very easy to distinguish the dura and to ascertain its relationship to the gland. It is apparent that the dura completely encapsulates the hypophysis and is adherent to it, without the intervention of the subarachnoid space or the leptomeninges. The latter are confined solely to that portion of the hypophyseal stalk located between the base of the brain and the diaphragm of the sella. These observations afford substantiating evidence for the conclusion reached in a previous study of other mammals (Wislocki, '37 a), to the effect that the subarachnoid space and typical leptomeninges do not surround the body (intraseellar portion) of the pituitary gland.

In conclusion a few remarks are necessary concerning the occurrence of melanophores in the hypophyseal region of the two-toed sloth and anteater. We attach no significance, as regards the hypophysis, to the presence of melanophores in the meninges surrounding the pituitary gland. In our experience melanophores are frequently encountered in variable numbers in the meninges of mammals, especially in the basal region. For example, in the pig and sheep the meninges are macroscopically black in appearance over large areas, while in the cat and rhesus monkey, although seldom visible grossly, melanophores can frequently be seen upon microscopic examination in the meninges at the base of the brain. These pigmented cells tend to occur in greatest numbers in the adventitial tissue accompanying the vessels coursing through the meninges.

Melanophores, in animals in which they occur in the meninges, are frequently seen in sections of the hypophyseal region. Here they may actually penetrate the tissues of the hypophysis, as observed by Benjamin ('35) in the rat, and in the present specimens of the two-toed sloth. This is quite natural and has no special significance beyond the fact that the stroma of the pituitary gland is derived embryologically from the perimedullary mesenchyme of the brain (Benjamin, '35; Wislocki, '37 b). The meninges and the hypophyseal stroma arise from a common formative tissue and consequently have similar histological characteristics. Benjamin has shown that only a certain proportion of rats shows this pigmentation and that the pigment has a hereditary basis. Furthermore, he points out that there is no evidence that the phenomenon is related in any way to the functioning of the hypophysis or that it is concerned in the relations of the pituitary to other glands of internal secretion.

CONCLUSIONS

1. The adult hypophysis of neither the three-toed sloth (*Bradypus griseus*) nor the two-toed sloth (*Choloepus hoffmanni*) possesses a pars tuberalis. A well-developed pars intermedia is present in both. The infundibular recess is extremely extensive.

2. In the adult anteater (*Tamandua tetradactyla*), a pars tuberalis is demonstrable on histological grounds, but its topographical relations are quite atypical. A pars intermedia is elaborately developed. The infundibular recess is extensive.

3. The adult armadillo (*Dasypus novemcinctus*) possesses a well-developed, typical pars tuberalis. It has, on the other hand, no pars intermedia. The infundibular recess is slight.

LITERATURE CITED

- BENJAMIN, T. A., JR. 1935 The occurrence of pigment in the pars intermedia and pars tuberalis of the hypophysis and in the hypophyseal leptomeninges of the rat (domestic and wild). *Anat. Rec.*, vol. 61, pp. 331-338.
- CAMERON, G. R. 1929 Die Beziehungen der Pars tuberalis hypophysis zum Hypophysenapparat. *Veröff. a. d. Kriegs- u. Constitutionspath.*, Bd. 5, S. 1-56.
- VALSÖ, J. 1934 Der Hormongehalt der Hypophyse des Blauwals (*Balaenoptera sibbaldii*). *Klin. Wochenschr.*, Bd. 13, S. 1819-1821.
- WISLOCKI, G. B. 1929 The hypophysis of the porpoise (*Tursiops truncatus*). *Arch. Surg.*, vol. 18, pp. 1403-1412.
- 1937 a The meningeal relations of the hypophysis cerebri. I. The relations in adult mammals. *Anat. Rec.*, vol. 67, pp. 273-294.
- 1937 b The meningeal relations of the hypophysis cerebri. II. An embryological study of the meninges and blood vessels of the human hypophysis. *Am. J. Anat.*, vol. 61, pp. 95-129.
- WISLOCKI, G. B., AND E. M. K. GEILING 1936 The anatomy of the hypophysis of whales. *Anat. Rec.*, vol. 66, pp. 17-41.

ABBREVIATIONS

ar, arachnoid membrane	mb, mammillary body
cf, colloid follicle	mr, mammillary recess
d, dura	op, optic chiasma
dc, dural capsule	pd, pars distalis
di, diaphragm of sella turcica	pt, pars tuberalis
int, pars intermedia	re, small residual cavities of infundibular recess
ip, infundibular process	rl, residual lumen of Rathke's pouch
ir, infundibular recess	sa, subarachnoid space
is, infundibular stalk	sd, subdural space
lm, leptomeninges covering hypophyseal stalk and tuber cinereum and containing blood vessels and melanophores but no epithelial cells	te, median eminence of tuber cinereum
	v, third ventricle

PLATE 1

EXPLANATION OF FIGURES

1 Medial sagittal section through hypophysis of two-toed sloth. There is no extension upward of epithelial tissue on the surface of the infundibular stalk or tuber cinereum. The epithelial pituitary is situated completely beneath the sellar diaphragm (di). The blackish tongue of tissue (lm), situated on the surface of the rostral aspect of the infundibular stalk, which might be assumed at this low magnification to consist of pars tuberalis, is composed solely of leptomeninges which stain darkly because of the presence of numerous blood vessels as well as melanophores. $\times 10$.

2 Parasagittal section through hypophysis of two-toed sloth. Note that the epithelial hypophysis lies within the confines of a stout dural capsule. $\times 10$.

3 Medial sagittal section through hypophysis of another two-toed sloth. Vascular injection with India ink. Notice in this specimen the small residual lumen which separates a clearly defined strip of pars intermedia from the anterior lobe. By virtue of the presence of blood vessels injected with India ink, the border between the buccal hypophysis and the neurohypophysis does not appear to be so sharply defined as in the previous specimen. The blackish masses seemingly penetrating the anterior border of the infundibular stalk are not upward extensions of cells from the buccal hypophysis but are injected blood vessels. No pars tuberalis is present. $\times 14$.

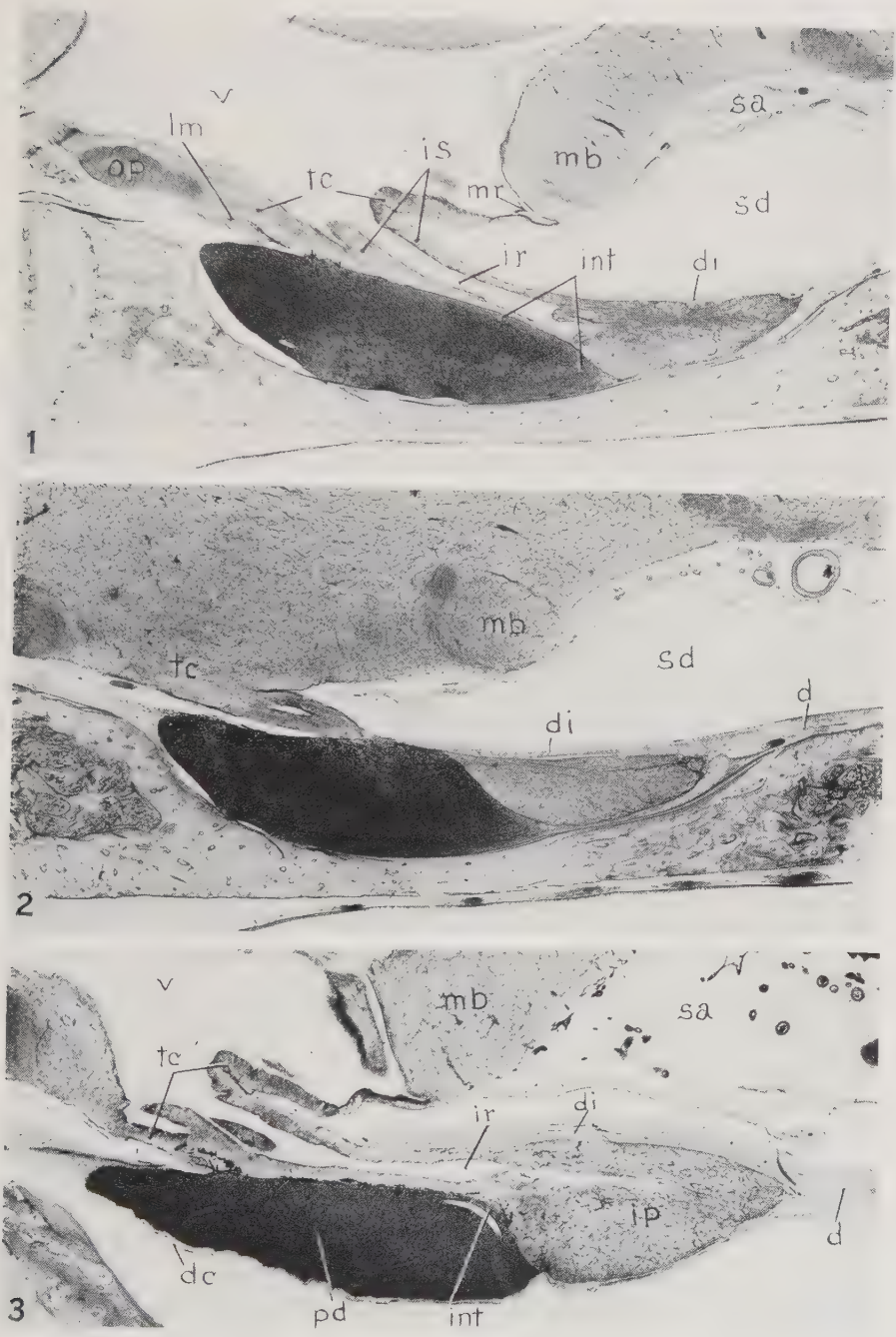


PLATE 2

EXPLANATION OF FIGURES

4 Medial sagittal section through hypophysis of three-toed sloth. No pars tuberalis is present. The pars intermedia is extensive. A stout dural capsule, including an extensive diaphragm, envelops the hypophysis. The buccal hypophysis possesses no extension upward along the infundibular stalk. $\times 10$.

5 Paramedian section through hypophysis of three-toed sloth, showing how, laterally to the infundibular stalk, the pars distalis and pars intermedia turn upward to form flanges which create a trough in which the stalk rests. $\times 10$.

6 Medial sagittal section through hypophysis of another three-toed sloth. Vascular injection with India ink. Infundibular stalk torn across. The extensive pars intermedia, the thick dural diaphragm and the long infundibular recess are clearly seen. $\times 10$.

7 Parasagittal section of the preceding specimen, taken somewhat more laterally than the section shown in figure 5. $\times 10$.

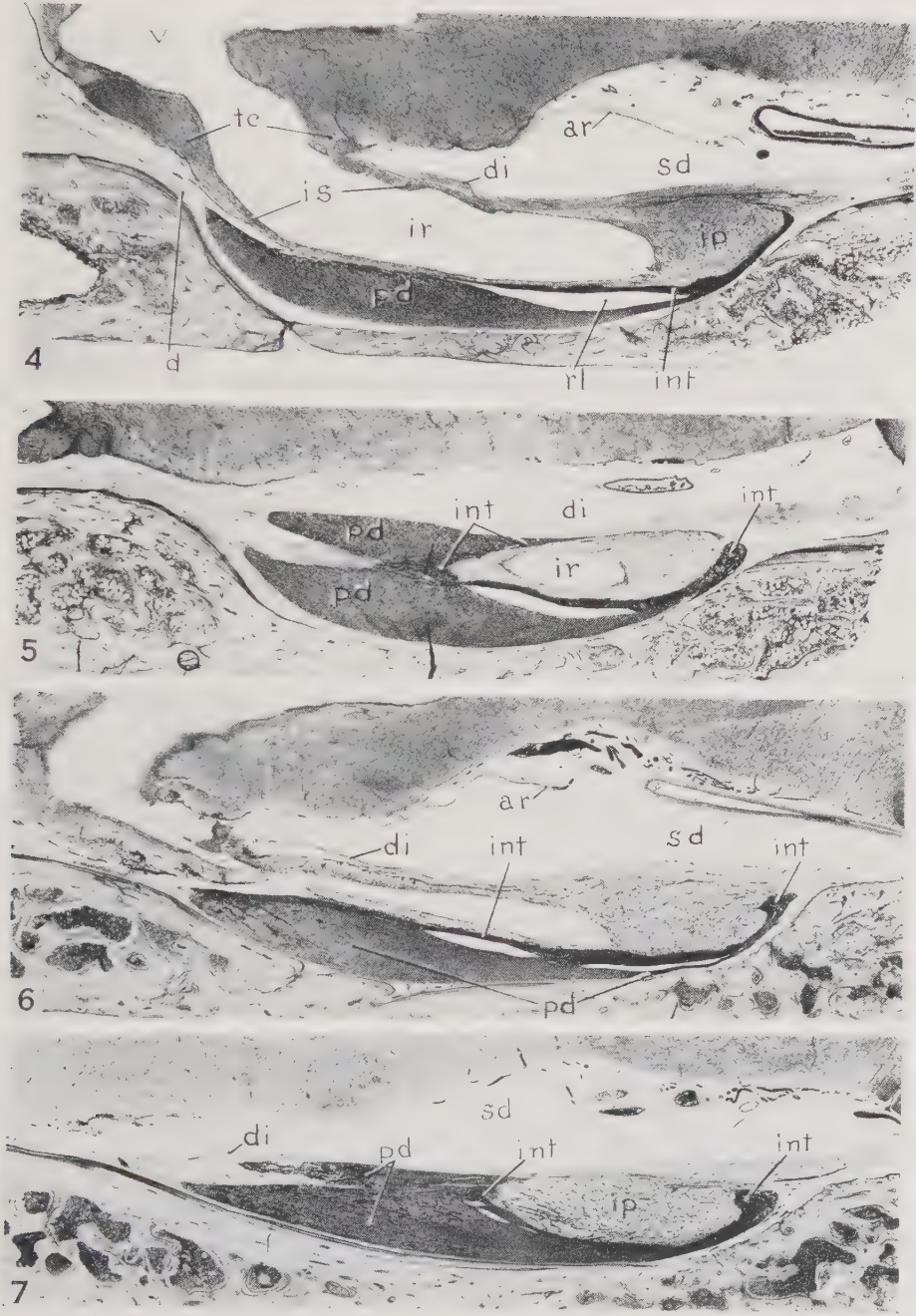


PLATE 3

EXPLANATION OF FIGURES

8 Median sagittal section through hypophysis of anteater. There is no pars tuberalis on the surface of the tuber cinereum (tc). The dark tissue (lm) in this region is composed solely of richly vascular leptomeninges which contain many melanophores. The bridge of the pars distalis, encircling the infundibular stalk, and its relationship to the pars intermedia (int) are shown in this and the following figure. The infundibular recess (ir) extends as an ill-defined cleft into the proximal part of the neural stalk. More distally there are situated in the substance of the stalk minute cavities apparently not continuous with the infundibular recess. The largest of these isolated clefts (rc) are barely visible at the magnification used here. $\times 10$.

9 Paramedian section of the hypophysis of anteater showing further details of the bridge of pars distalis (pd) surrounding the neural stalk. The extensive and complex pars intermedia (int) can also be seen. Noteworthy is the most rostral part of the intermedia in the paramedian position. Labelled 'pt' in the figure, we regard this anterior extension of the intermedia, on histological grounds, as equivalent to the pars tuberalis of other mammals. $\times 10$.

10 A section taken still more laterally than the preceding one. It shows how the separate bridge-like mass of pars distalis in the two previous photographs is actually connected with the lateral portions of the anterior lobe. $\times 10$.

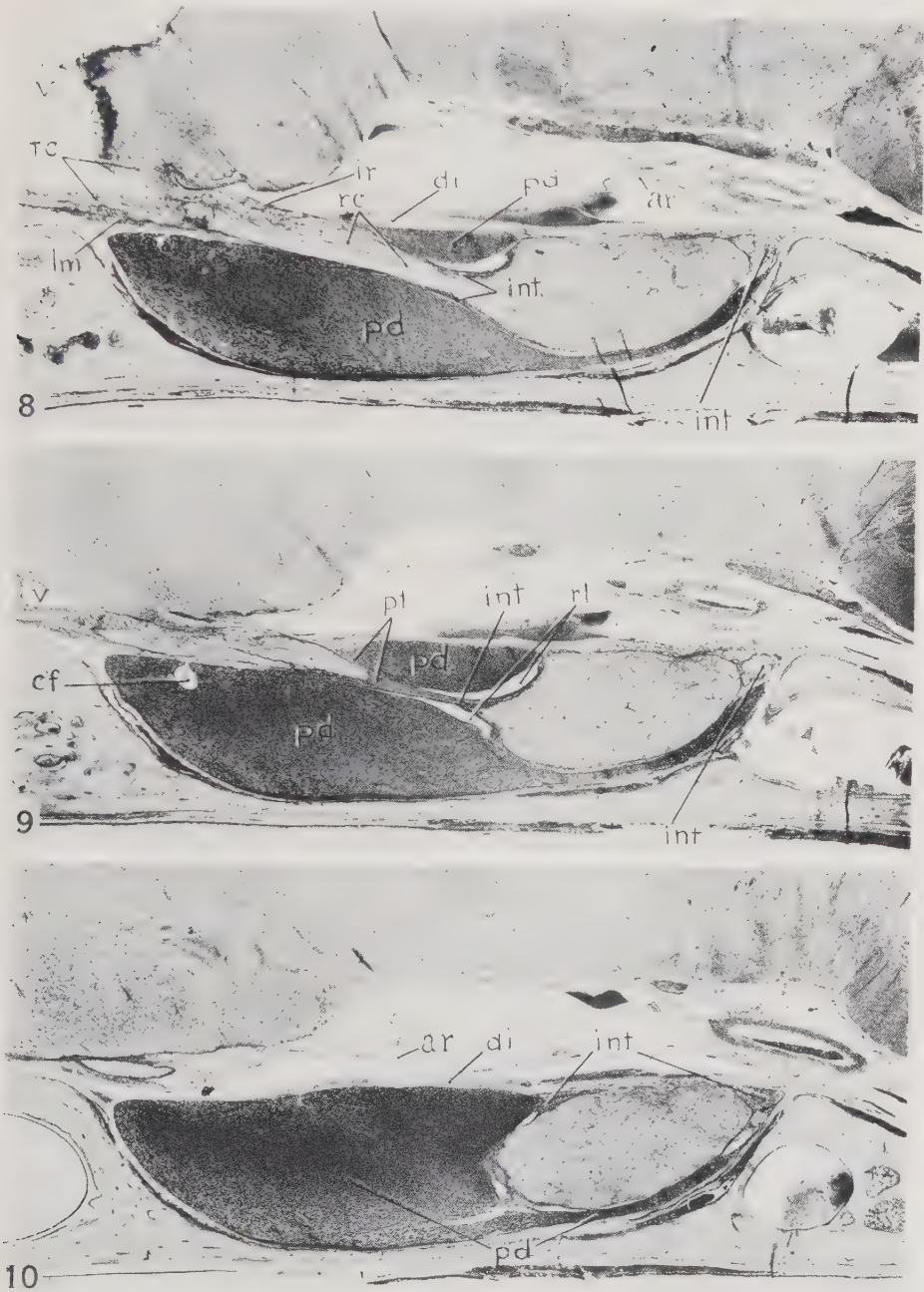
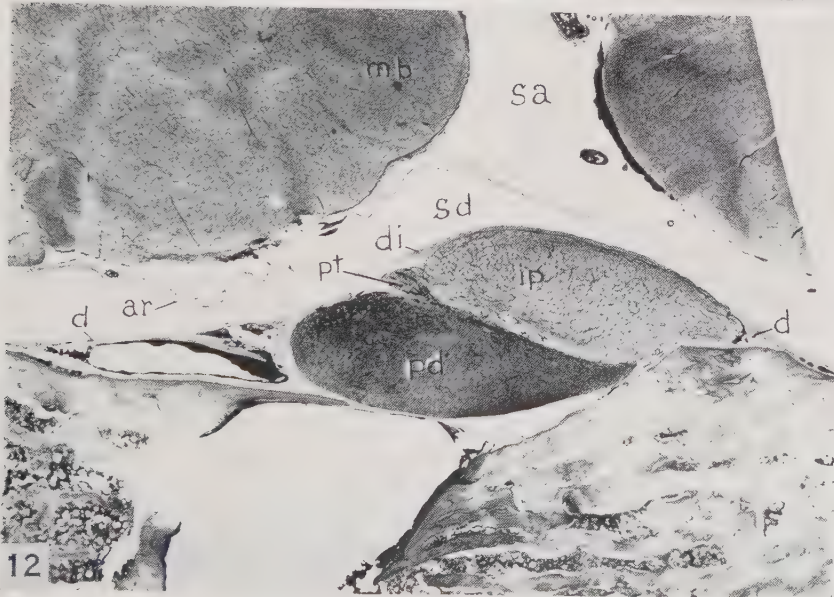
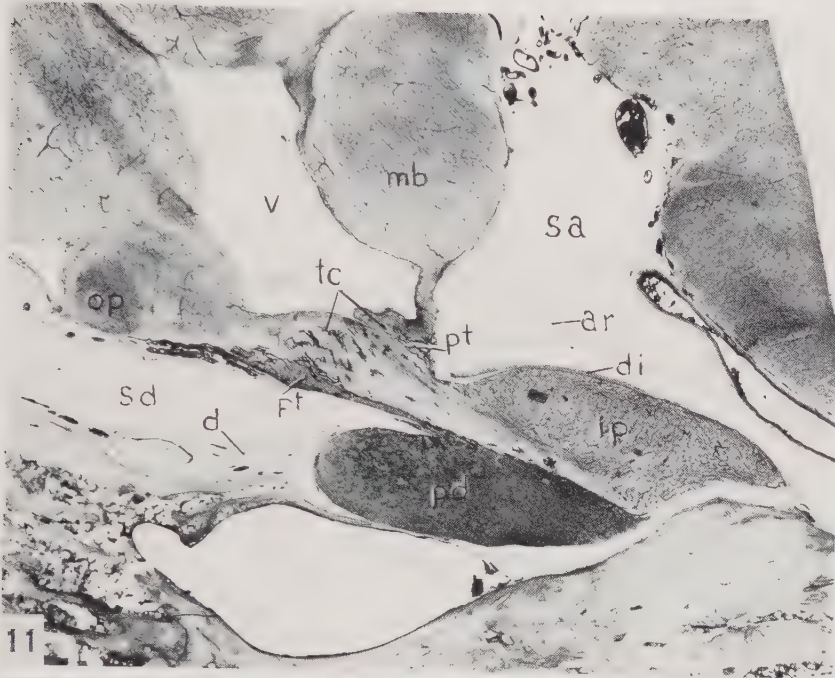


PLATE 4

EXPLANATION OF FIGURES

11 Medial sagittal section of the hypophysis of armadillo. Vascular injection with India ink. Noteworthy is the extensive pars tuberalis (pt) and the total absence of a pars intermedia. The line of demarcation between the epithelial hypophysis (pd) and the neurohypophysis (ip) is very sharp, histological examination indicating the presence of a delicate and distinctive lamina of capsular connective tissue and blood vessels separating the two. $\times 14$.

12 Paramedian section of the hypophysis of armadillo. In this and the preceding photographs the topography of the dura surrounding the gland is well shown. It will be observed that the intimate encapsulation of the gland by the dura precludes the extension of the subarachnoid space around the body of the gland; the leptomeninges, as far as they exist as individual layers enclosing the subarachnoid space, terminate as a collar around the hypophyseal stalk. $\times 14$.



A CYTOLOGICAL STUDY OF FATIGUED MUSCLE

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ONE PLATE (FOUR FIGURES)

INTRODUCTION

Luna ('11), Beams ('29) and others have described the Golgi material in striated muscle fibers as situated in the sarcoplasmic space at each pole of the muscle nucleus. According to Maximow's Histology, the mitochondria are located in the interfibrillar sarcoplasm and at the nuclear poles in striated muscle fibers.

Apparently little or no study has been made of these structures in muscle fibers in various physiological states. Therefore, in this investigation the Golgi material, the mitochondria and other cytoplasmic inclusions were studied in skeletal muscle fibers from fatigued and resting white rats.

MATERIALS AND METHODS

The Kolatchev Golgi technique, as modified by Nassonov, and the Bensley mitochondrial method were used. Four-week-old male white rats were employed. Tissues from four normal and four fatigued animals were studied.

The rats were fatigued by running. The track provided was a round box lined with corrugated paper and mounted on an axle. The box was revolved by a motor. After 90 or more minutes of running at moderate speed, the rat fell exhausted and was immediately killed.

Natural gas was used to kill the animals. Small pieces of limb muscle were removed to the fixative, then washed in running water, rinsed in distilled water and rapidly brought

up through the alcohols into cedar oil. After at least 24 hours in the cedar oil, they were embedded in paraffin. Sections were cut at $4\ \mu$, and mounted on the slide by the gelatin-formol method of Haupt.

OBSERVATIONS

Examination of the Golgi material in skeletal muscle fibers from the resting rats revealed, in agreement with Macdougald ('36), that this material is in the form of an apparently homogeneous, small, more or less spherical body placed in the sarcoplasmic space at each pole of the muscle nucleus directly in contact with or a very short distance from the nuclear membrane (fig. 1). This compact polar body was never bisected in the rat as I have observed it in many Golgi preparations of resting guinea pig and rabbit skeletal muscle. Also, the bodies at the nuclear poles could not be observed to have a reticular or net-like structure as my preparations of frog skeletal and cardiac muscle have shown them to possess or as Luna ('11) found in his investigations on the Golgi apparatus in vertebrate cardiac muscle. In rat skeletal muscle the Golgi material was never found along the sides of the nuclei as described by Eastlick ('37) in vertebrate cardiac muscle, nor was it ever in the form of large pyramidal masses like those observed by Beams ('29) in cockroach striated muscle.

In muscle fibers from the fatigued rats the Golgi body at each pole of the muscle nucleus was seen to be finely divided, the fragments being more or less dispersed (fig. 2). The total quantity of Golgi material represented by these fragments was roughly that which would be expected from the size of the single Golgi body of the resting fiber. In a few instances this granular condition was encountered in the resting rat muscle fiber; in the fatigued fiber, however, it was consistently observed. Occasionally, the compact Golgi body, characteristic of the resting state, was observed in fibers from a fatigued rat.

The rather large spherical mitochondria in the fatigued as well as in the unfatigued skeletal muscle fibers were in the sarcoplasmic space at each pole of the nucleus or among the myofibrillae. Typically, in a given fiber, fatigued or not, the mitochondria were not observed in the polar and interfibrillar positions simultaneously. These bodies were not distinguishable from the sarcosomes (J and Q granules), if these were present in the interfibrillar sarcoplasm. For this reason, the morphology of the mitochondria at the nuclear poles was used for comparison in the fatigued and resting muscle fibers.

It was found, in agreement with Feyel ('37), that the mitochondria had not changed in size, shape or distribution in response to muscle fatigue (compare Lewis, '26). Mitochondria, at each pole of the muscle nucleus, are shown in figure 4. When the mitochondria at the nuclear poles were demonstrated with osmic acid (fig. 3), their morphology and position in the fatigued and unfatigued muscle fibers were identical. The mitochondria thus blackened suggest that the polar bodies described by Merland ('34) in mammalian cardiac muscle as 'Golgi vacuoles' were possibly mitochondria. This formation was not observed often in my Golgi preparations, a result which was to be expected in that the mitochondria are only occasionally impregnated by the Kolatchev method. In addition, these blackened bodies were seen among the myofibrillae as was the case in the mitochondrial preparations. Against the interpretation that these are mitochondria is the fact that Merland was able to demonstrate them, or similar bodies, by supravital neutral red technique.

In both the Kolatchev and Bensley preparations numerous granules of varying sizes were often impregnated or stained in the general sarcoplasm. For the most part these were definitely J and Q granules and some may have been mitochondria or fat droplets (compare Bullard, '12). Often the granules—which were always spherical—had no definite arrangement. The quantity and appearance of these bodies in the fatigued fibers did not differ from that observed in the unfatigued fibers.

In the Golgi preparations the sarcoplasm was sometimes deeply blackened; Cohnheim fields were thus clearly defined. Muscle fatigue did not change the intensity or manner of this impregnation.

DISCUSSION

The form and distribution of the Golgi material in muscle fibers is different from that found in most other kinds of cells, and appears to be a response to spatial requirements in this tissue. The bipolarity of the Golgi material in muscle is comparable to the perinuclear position of the Golgi apparatus in nerve cells. This arrangement suggests that in both kinds of cells the Golgi material may play its physiological role more generally through the cytoplasm than it does in other kinds of cells where it is confined to one side of the nucleus. The relatively small quantity of Golgi material in muscle would perhaps indicate that the material has a limited role in muscle physiology. Eastlick ('37) has observed in vertebrates that there is relatively more Golgi material in cardiac muscle than there is in skeletal muscle; according to my observations on the rat, however, there is no greater amount of Golgi material present at each nuclear pole in cardiac muscle than there is in skeletal muscle from this animal. Also, I have observed the identical size of the Golgi reticulum in frog cardiac and skeletal muscle. Frog smooth muscle possesses a very extensive perinuclear Golgi network; it is difficult to correlate this relatively large amount of Golgi material with any physiological activity peculiar to smooth muscle tissue in the frog. In developing striated muscle there is always more Golgi material than there is in the adult tissue.

The fragmented condition of the Golgi body observed in the fatigued muscle fibers is in agreement with the generally accepted theory that the Golgi material in an active cell is always in a more dispersed state than it is in the resting cell. Changes in the morphology of the Golgi material have been correlated with secretory activity in gland cells by Bowen ('29) and others. The fragmentation of the Golgi body in

fatigued skeletal muscle cannot be correlated with any known secretory activity in this tissue. The dispersed state of the Golgi material here can only be taken to be indicative of a heightened physiological state. An analogous situation is encountered in the neuron where, according to Penfield ('20), the Golgi net breaks up (retispersion) in response to trauma and faradic stimulation.

The mitochondria in the adult muscle fiber appear to be passive to the physiological state of the muscle. Mitochondrial participation in myofibril development is not established. The function of these bodies in muscle can only be conjectured.

The miscellaneous granules in the general sarcoplasm also seem to be passive to muscle fatigue. As far as could be discerned, in a rough quantitative way, these bodies—J and Q granules and fat droplets—had not been affected by muscle fatigue. Some authors (Cohn, '28) believe that the granules and droplets are stored food. That they appeared to be unaffected either in amount or form in this investigation, may be taken as evidence against this interpretation. These bodies were found closely packed in large quantity in the fatigued as well as in the unfatigued fibers, and never appeared diminished in size or number. They often were blackened along with the Golgi material—and I have frequently observed this also in rat cardiac muscle—or were stained along with the mitochondria. Often in the skeletal muscle preparations the Golgi material or the granules alone were impregnated. Although the osmic acid method did not differentiate the granules from the Golgi material in a very specific manner, other and less complex techniques show that the granules are sarcosomes, fat droplets or mitochondria. According to Bullard ('12), only fat droplets containing unsaturated fatty acids reduce osmic acid. Mitochondria may be impregnated after both the Kolatchev and Weigl Golgi techniques.

The osmic acid method of Kolatchev does not indicate any difference, chemically or otherwise, between the sarcoplasm of the fatigued muscle fiber as compared with that of the unfatigued fiber. Obviously, a more specific technique would be needed to determine any such differences, if they exist.

SUMMARY

1. The Golgi material fragments in response to muscle fatigue.
2. The mitochondria show no response to muscle fatigue.

LITERATURE CITED

- BEAMS, H. W. 1929 Studies on the 'Golgi apparatus' of insect muscle. *Anat. Rec.*, vol. 42, pp. 323-332.
- BOWEN, R. H. 1929 The cytology of glandular secretion. *Quart. Rev. Biol.*, vol. 4, pp. 299-324; 484-519.
- BULLARD, H. H. 1912 Granules and fat of striated muscles. *Am. J. Anat.*, vol. 14, pp. 1-46.
- COHN, A. E. 1928 Cardiac Muscle, in *Special Cytology*, E. V. Cowdry.
- EASTLICK, H. L. 1937 A cytological study of the Golgi substance of striated muscle of vertebrates. *J. Morph.*, vol. 61, pp. 399-427.
- FEYEL, THÉRÈSE 1937 Sur le chondriome de la fibre musculaire striée chez la grenouille. *Compt. rend. Soc. de biol.*, T. 124, pp. 1048-1050.
- LEWIS, W. H. 1926 Cited by H. W. Beams, 1929.
- LUNA, E. 1911 Sulla fine struttura della fibra muscolare cardiaca. *Arch. f. Zellforsch.*, vol. 6.
- MACDOUGALD, T. J. 1936 The Golgi apparatus in the fibres of cardiac muscle. *Zeit. f. Zellforsch. u. mikr. Anat.*, vol. 24, pp. 399-410.
- MAXIMOW-BLOOM A Text-Book of Histology, First ed. W. B. Saunders Co.
- MERLAND, A. 1934 Appareil de Golgi de la fibre musculaire cardiaque chez quelques mammifères. *Compt. rend. Soc. de biol.*, T. 115, pp. 1647-1648.
- PENFIELD, W. G. 1920 Alterations of the Golgi apparatus in nerve cells. *Brain*, vol. 43, pp. 290-305.

PLATE

PLATE 1

EXPLANATION OF FIGURES

Spencer camera lucida. All figures: $\times 1350$; reduced about $\frac{2}{3}$.

1 Golgi material in bipolar position. Resting rat skeletal muscle. Kolatchev technique.

2 Golgi material in bipolar position. Fatigued rat skeletal muscle. Kolatchev technique.

3 'Golgi vacuoles' or mitochondria (?) in bipolar position. Resting rat skeletal muscle. Weigl technique.

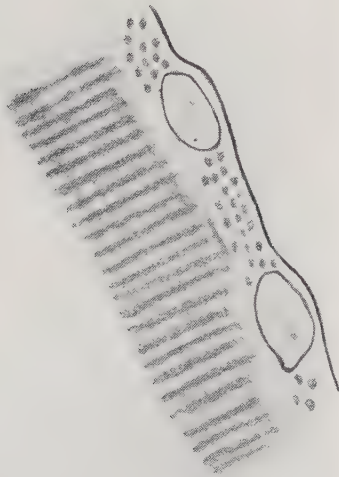
4 Mitochondria in skeletal muscle. Four-week-old resting rat. Bensley technique; aniline acid fuchsin-methyl green stain.



2



3



4

THE DEVELOPMENT OF CRYPTS IN THE HUMAN PALATINE TONSIL¹

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SEVENTEEN FIGURES

INTRODUCTION

Although much has been written concerning the development and morphology of the human palatine tonsil as a whole, yet little stress has been placed on the development and morphology of its crypts. Gulland (1891), Stöhr (1891) and Kollman ('00) briefly considered the development of crypts, but did not furnish any adequate details. The results of Hammar ('03) are intimately related to the present problem. Among the models of human palatine tonsils constructed by Hammar occur stages comparable to some of those in the present series. Although Hammar did not describe the crypt systems in detail, his illustrations serve as valuable aids for comparison. It is the purpose of this paper to describe and discuss some of the more important features concerning the developing crypt systems as determined from a study of serial sections and wax models of human palatine tonsils.

MATERIAL AND METHODS

Serial sections were prepared from thirty-one normal specimens ranging in age from the beginning of the third lunar month to term. An additional postnatal specimen 1 month

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I wish to take this opportunity to acknowledge my appreciation to Dr. L. B. Arey for his invaluable aid in this investigation and in the preparation of the manuscript.

old was modelled; although death had followed lobar pneumonia, the tonsils were judged normal. The freshly obtained material was fixed in formalin, sectioned serially and stained with haematoxylin and eosin. In most instances both tonsils of each specimen were prepared in this manner. All the series received careful microscopic study, and seven wax reconstructions (at 75 to 100 times linear) were made of five significant stages in crypt development. The lunar-month age and crown-rump lengths of the specimens modelled are as follows: 100 mm. (3.5 months); 130 mm. (4.2 months); 210 mm. (6.0 months); 230 mm. (6.5 months); 340 mm. (10 months); ? mm. (1 month postnatal). In tracings made with an Edinger projection apparatus the basal layer of epithelial cells was followed so as to include the solid ingrowths as well as those variously hollowed. The crypts on the resulting models were then counted and their form, relationships and peculiarities both observed and recorded. In counting and classifying the crypts it was held that any primary invagination from the tonsillar fossa is a first-order crypt. Similarly, a second-order crypt was considered as any branch of a first-order crypt, and so on. Crypts appended by a narrow neck were classified as vesicles. In addition, information was obtained on the development, position, and relation of the peritonsillar mucous glands and their points of drainage.

TONSILLAR FOSSAE AND PLICAE

The plica supratonsillaris is present in all the specimens modelled; it is more prominent in the later stages than in the 3.5- and 4.2-month models. The plica triangularis is also constant throughout the period studied. Separation into anterior and posterior fossae is indicated in the stages at 3.5 and 4.2 months, although no distinct plica intratonsillaris (Hammar) can be found. Hammar demonstrated the bilobed nature of the tonsil by modelling the lymphoid tissue. This bilobed condition is not so readily recognizable when the epithelium only is modelled. The anterior fossa exceeds the posterior fossa in size and in the complexity and number of its in-

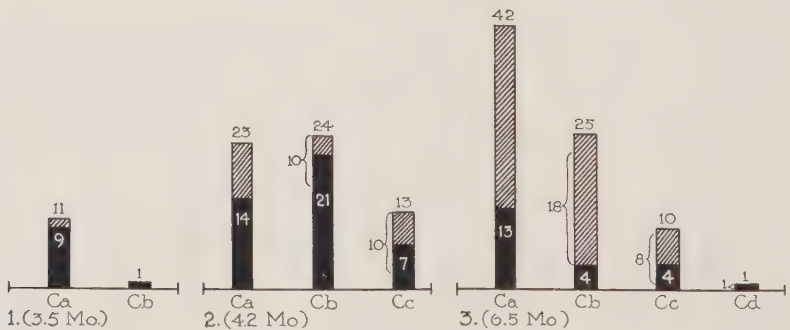
growths. There were no retrotonsillar plicae, so that in all instances the tonsillar fossa were not marked off sharply from the pharyngeal wall.

TONSILLAR CRYPTS

The development of the crypt system dates from the third month. Arising from the lateral wall of the tonsillar fossa in the 3.5-month model there are three long, tubular ingrowths which curve downward and outward, and eight lesser, simple, bud-like ingrowths, the largest of which is associated with the supratonsillar recess (fig. 13). The first of the tubular ingrowths just mentioned arises from the supratonsillar fossa, the second from the midregion of the fossa, and the last (which bears a single, solid, second-order branch) from the lower part of the fossa. The mouths of two of the larger, more extensive ingrowths have already formed by desquamation of the central cells most remote from the basal layer. These central cells of an epithelial ingrowth are larger, paler and older than the basal cells, and terminate their life cycle by desquamation. The 'hollowing-out' process has taken place without the desquamated cells having compacted into an epithelial plug, as will be described for other parts of the crypt system. It is probable that some of these ingrowths, so created, are absorbed, wholly or in part, into the growing, expanding wall of the tonsillar fossa. As a whole, the crypt system at this stage is relatively insignificant (figs. 1 to 3). The epithelium is characterized at its actively proliferating basal border by several layers of apparently similar cells which are not sharply demarcated by a basement membrane from the surrounding mesenchyme. All of the rapidly growing portion of an invading process shows epithelium of this character.

In the 4.2-month model the epithelium of the fossal wall that is associated with the epithelial ingrowths is greatly thickened, due to the burst of proliferative activity which characterizes this period of tonsillar development. This thickening of the epithelium is not general, but is confined to irregular, local

areas which represent the expanded roots of epithelial ingrowths. The lateral wall and deeper recess of the tonsillar fossa are dotted with numerous pits and deeper recesses of varying caliber and depth; these indicate mouths of first-order crypts which are in various stages of lumen formation. This model shows many simple bud-like ingrowths and seventeen rounded or oval, cylindrical, first-order epithelial ingrowths of varying length and diameter (fig. 14). These latter, first-order ingrowths take an approximately parallel course in an inferior direction. Eleven of such first-order ingrowths have in all twenty-four second-order branches arising from them.

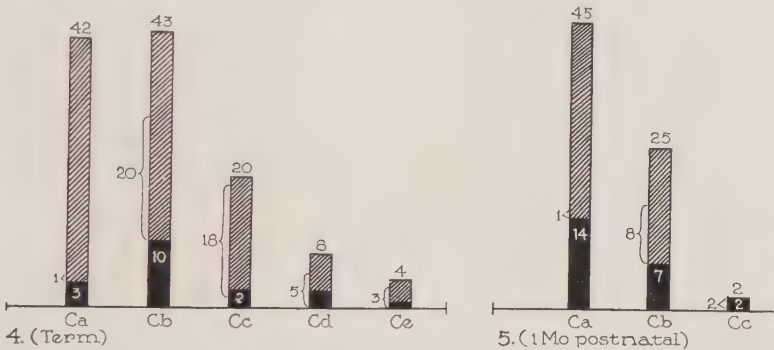


Figs. 1 to 3 Graph showing the numbers of crypts in models 3.5, 4.2 and 6.5 months. Note the gradual increase with age in the number and order of crypts; also the increase in the number of vesicles. Ca., first-order crypt; Cb., second-order crypt, etc. Solid epithelial ingrowths, in black; other crypts, hatched. Numbers opposite brackets refer to the number of crypts which are vesicles. Total numbers of crypts are indicated at the top.

Nineteen of the second-order ingrowths also project in an inferior direction, but three extend approximately at right angles to the first order ones, and two in a superior direction. Of the thirteen third-order invaginations, five project inferiorly and five superiorly. Study of later stages indicates that these downward growing crypts and their branches, which represent the major part of the crypt system at this period, are destined to atrophy and disappear. Even at the present period the beginning of this process is indicated by the fact that twenty-one crypts have constricted attachments while

still others give indications of constriction. Reference should be made to figures 1, 2, 13 and 14 in order to appreciate the advancement of the crypt system during the period (slightly over 2 weeks) involved.

The tonsil at 6.5 months reveals a tonsillar sinus differing in no essential detail from that of the preceding stages. The crypt system (fig. 3) shows double the number of first-order crypts, most of which have hollowed out. The number of second- and third-order crypts remains practically unchanged, but most of the second-order crypts now present lumina. One fourth-order bud, a small prevesicle, is present. It is to be



Figs. 4 and 5 Graph showing the numbers of crypts in models at term and 1 month after birth. Note the increase in number and order of crypts and number of vesicles in the term model over the 6.5-month model. Note also the decrease in the number and order of crypts and number of vesicles in the 1-month postnatal model as compared with the term model. This difference is accounted for by the virtual disappearance of many vesicles. Ca., first-order crypt; Cb, second-order crypt, etc. Solid epithelial ingrowth, in black; other crypts, hatched. Numbers opposite brackets refer to the number of crypts which are vesicles. Total numbers of crypts are indicated at the top.

noted that the second-, third- and fourth-order crypts have narrow, constricted necks. Such crypts with constricted necks will be called vesicles, or, if solid, prevesicles (compare figs. 6 a to 8 a). The essential difference between the crypt system at 4.2 and 6.5 months lies in the fact that in the latter there are many ingrowths without constricted attachments. These represent the future, permanent crypts; among them occur

the large, flat, plate-like type (straight or curved), such as are characteristic of the models of the adult tonsil. The model prepared of the 6.3-month stage shows no important differences from the 6.5-month stage just described.

Examination of the model of the tonsil at term reveals a more advanced crypt system than that found in the preceding 6.5- and 6.3-month specimens (figs. 4 and 16). The straight and curved, plate-like type of crypt now predominates both in number and in size. A few crypts of the second- and third-order, now more advanced, also have assumed this shape. The number of first-order crypts is unchanged; on the other hand, the number of second-order crypts has increased nearly two-fold, many in the form of epithelial vesicles, as in the 6.5-month specimen. Similarly, the number of third-order crypts has doubled; almost all of these are epithelial vesicles. Of the twelve fourth- and fifth-order crypts of this specimen, eight are vesicles or prevesicles. The second model at term is in all essentials like the one just described.

Examination of the model prepared from an infant 1 month old revealed an astonishing reduction in the total number of crypts (figs. 5 and 17). Again, the number of first-order crypts remains constant. The straight or curved, plate-like type of crypt predominates both in number and in size. The second-order crypts are reduced almost one-half as compared with the same structures 1 month earlier. The number of third-order crypts is negligible (two) and no fourth- or fifth-order crypts are to be found. This difference is accounted for by the fact that most of the attached vesicles and crypts, characteristic of the preceding stages, have finally been cut off from the permanent crypt system and have either completed their course of atrophy and degeneration or consist of mere remnants. Proof of this progressive degeneration of part of the crypt system can be had by a microscopic study of the tonsil at various typical stages in its development.

GROWTH OF THE CRYPT SYSTEM

Stöhr (1891) discussed the growth of crypts in the human palatine tonsil briefly and Minot (1892) incorporated Stöhr's observation in his text on human embryology. The account as found in Minot is as follows: "The formation of solid tonsillar buds continues not only through the embryonic period, but also for a year after birth. The solid buds gradually become hollow by a change in the central cells, which assume a corneous appearance and gradually contract into a mass in the centre of the bud, until it communicates with the space containing the degenerated mass which is then expelled."

Kingsbury and Rogers ('27) in discussing formation of crypts in the palatine tonsil of the calf mention that the sprouts remain solid in embryos as large as 265 mm. Later (445 mm.), these cords develop lumina which communicate with their respective fossae. Also at this time the majority of the sprouts have hollowed out by a process of degeneration in their central cells. They found that the growth of the distal part of the epithelial cords exceeded the growth of the proximal part, and the enlarged, bulb-shaped end-piece had a correspondingly large lumen. No portions of the epithelium were seen to separate and undergo degeneration in this animal. It would seem that this type of degenerative process is a peculiarity of the development of the human palatine tonsil; at least, it has not been recorded for lower animals.

Levin ('30), reporting on the development of the tonsil in the domestic pig, states that the first signs of crypt formation is at the 142 mm. stage. He writes: "Cells at the center of the epithelial outgrowths enlarge with the formation of the pink-staining cytoplasm (keratinization?). This process of cell transformation extends to the surface."

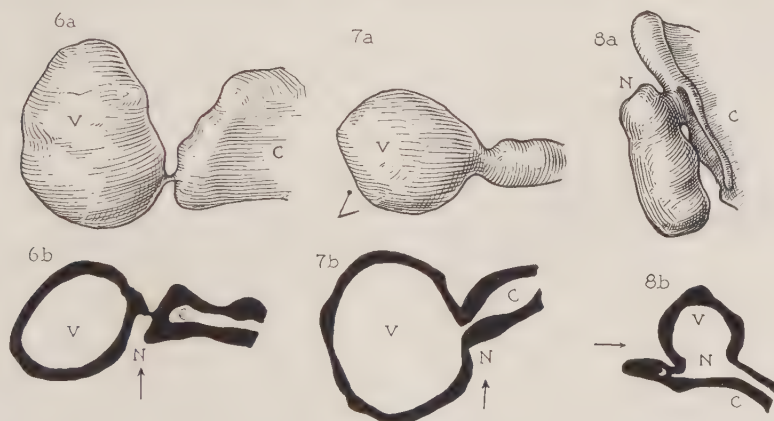
Hammar ('03), in his discussion concerning the histogenesis of the human palatine tonsil, mentioned certain 'cut-off epithelial balls' and pointed out that these structures were not precursors of lymphocytes or secondary follicles, as interpreted by Retterer (1888). He states in another paragraph

that some of these epithelial balls may atrophy and disappear under the influence of leucocytic invasion while others are transformed into epithelial vesicles filled with cellular debris.

In the human palatine tonsil degeneration of the central cells apparently takes place first in the actively growing parts of the epithelial ingrowth. Hence distal lumen-formation is common because the end of an epithelial process is often the region showing the most active growth. However, lumen-formation also occurs from the fossal wall distally and it is commonly in progress proximally and distally in the same crypt. The degenerated, central epithelial cells aggregate progressively into compact epithelial plugs. The relative activity of the basal cells in different portions of the epithelium determines the size and shape of the epithelial ingrowth. The position and order of a crypt are also determined by the characteristic method of growth employed. For example, a first-order crypt of the fourth month may later become a second-order crypt due to the appearance of a new ingrowth near the point of its attachment. The formation of crypts with constricted necks is brought about by a combination of rapid growth and central degeneration of the cells in one part of the epithelium while a more proximal part lags in development to become the solid neck. The round or oval, cylindrical ingrowths of the early stages tend to undergo constriction and vesicle formation. A study of the epithelial necks of vesicles reveals the solid neck to be most common, its width varying from a broad attachment to a narrow connection one to two cells across (figs. 6 a and 6 b). In the 6.5-month specimen there was but one vesicle, while in the term tonsil five vesicles were considered to have doubtful attachments and possibly were totally detached. Degeneration of the central cells in the neck to effect open communication with the parent crypt occurs less often. One such vesicle was found in the 6.5-month specimen, five at term and six in the 1-month postnatal stage. Figure 7 a shows a total drawing of such a vesicle from the term tonsil and figure 7 b a

section through its neck. Figures 8a and 8b similarly illustrate a vesicle with a broad neck which has completely hollowed out to produce a wide communication into a first-order, plate-like crypt. Such a vesicle will probably remain a part of the permanent crypt system. Vesicles with narrow, open necks tend to degenerate.

The period of vesicle formation will be best understood by noting the number of vesicles on each model. The 3.5-month model has no vesicles. The 4.2-month model has twenty-one



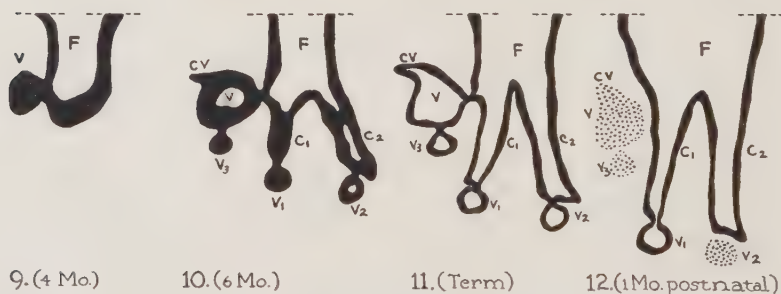
Figs. 6a to 8b 6a, a drawing of an epithelial vesicle (V) having a narrow, solid neck (N). 6b, a transverse section through the neck region of 6a. 7a, a drawing of an epithelial vesicle having a narrow, open neck which connects the lumen of the vesicle with the crypt system (C). 7b, a transverse section through the neck region of 7a. 8a, a drawing of an epithelial vesicle having a broad, open neck. 8b, a transverse section through the neck region of 8a. Vesicles with solid necks occur more commonly than those with open necks.

vesicles, only ten of which have hollowed into true vesicles, whereas eleven are solid prevesicles. The 6.5-month model has twenty-seven vesicles, seven only of which are prevesicles (the larger vesicles average 0.25 to 0.4 mm. in greatest diameter). The term model has forty-seven vesicles, four of which are prevesicles (the larger vesicles average 0.6 to 0.8 mm. in greatest diameter). The 1-month postnatal model has only eleven vesicles, two of which are prevesicles. It is probable that the number of prevesicles and vesicles increases

rapidly in the early stages of the developing crypt system; thereafter the increase is more gradual up to term.

A series of diagrams has been prepared in an effort to summarize some of the more important points of the growing crypt system (figs. 9 to 12). Figure 9 represents the thickened epithelium of the tonsillar sinus during the fourth month; a single, solid, first-order, tubular crypt (prevesicle) arises from its wall.

Figure 10 illustrates a similar section during the sixth month. The epithelial vesicle, V, has grown larger and its central cells have started to degenerate and thus form an epithelial plug. It now bears CV, a second-order crypt and VS,



Figs. 9 to 12 Diagrammatic representation of the developing crypt system to illustrate the formation and degeneration of vesicles and the various types of lumen formation. (Full explanation in text.)

a pre-vesicle. It still retains the narrow solid neck. Two new crypts (C₁ and C₂), of the flat, plate-like type, have also developed. C₁ is almost completely solid, but presents at its end a vesicle (V₁) which is beginning to hollow out. C₂, which shows both central and distal lumen formation, also presents a small vesicle (V₂) with a narrow, solid neck.

In figure 11 the large vesicle (V) has completely hollowed out, its lumen containing an epithelial plug and scattered lymphocytes. Nests of lymphocytes appear within parts of the thinning epithelium. This main vesicle has a small diverticulum (CV), and also a vesicle (V₃), appended by a narrow, solid neck. The flat, plate-like crypts C₁ and C₂ have their

lumina completed, C_1 by means of a progressive degeneration of cells proximo-distad (relative to the fossa) and C_2 by simultaneous proximal and distal lumen formation. The vesicles V_1 and V_2 have likewise completed lumen formation. The vesicles V_1 and V_2 have likewise completed lumen formation, but their necks are still solid. At this period, C_1 and C_2 are first-order crypts. Vesicle V , originally in relation to the sinus, now appears to arrive from the base of C_1 and conforms to our definition of a second-order crypt. CV and V_3 , appended to V , are now third-order crypts.

In figure 12, which represents a postnatal specimen, the large vesicle (V), with its diverticulum (CV), is completely cut off and degenerate. V_2 and V_3 have also degenerated and lost their attachments. The central cells in the neck of V_1 have degenerated to afford open communication with its mother crypt (C_1).

Degenerating epithelium is characterized by localized areas of vesiculation of epithelial cells and an interruption of epithelial continuity due to invasion of lymphocytes. In some places only a thin layer of flattened, desquamating cells remains. Numerous lymphocytes are found free in the vesicular lumen, along with much cellular debris. The degenerative process starts in the prenatal months, and in the present series had involved most of the vesicles in the term specimen. It is possible that some vesicles degenerate completely in the prenatal months, but no definite proof of this was found in the present series. In the specimen obtained 1 month after birth, almost all the vesicles were cut off and completely degenerated; few transitional stages remained. A narrowing of the neck region, due to a combination of growth changes and degeneration, is commonly responsible for the 'cutting off' of the vesicle. Whether the epithelial degeneration is primary or secondarily due to some other factor, such as the influence of lymphocytic invasion as mentioned by Hammar ('03), is not known. In this regard it might be mentioned that lymphocytes are commonly found migrating through epithelium, wherever lymphocytes and epithelium are associated, without resulting in any complete degeneration of

the epithelium so invaded. Other factors which might explain the degeneration were not apparent. The position of the completely degenerated vesicle is marked in sections by a relatively clear area devoid of vessels and fixed, connective-tissue elements. Degenerating epithelial cells can be recognized around the margins of this relatively pale area. The area itself contains lymphocytes, free cells of the reticular connective tissue, and often portions of the epithelial plug which previously occupied the lumen of the vesicle. In general, these clear areas are the same shape and size as the various vesicles found in the prenatal months. The degeneration of the epithelial wall leaves a space in the tonsillar tissue which is filled-in, first by the lymphocytes and other free cells of the reticular connective tissue and later by the ingrowth of the fixed, connective-tissue elements, blood and lymphatic vessels and connective-tissue reticulum. The meaning of this degenerative process is obscure. However, degeneration of portions of epithelium is found to occur in the developmental history of other organs, such as, for example, thymus, thyroid, parathyroid, liver and kidney.

LYMPHOID TISSUE

Lymphocytes are already present in the early part of the third lunar month as condensations at the tips and sides of the epithelial ingrowths and around the regional blood vessels. Typical diffuse lymphoid tissue is present in the fourth month. Numerous lymphocytes are seen in the large efferent lymphatic channels and in the smaller lymphatic radicals within the lymphoid tissue. Solid secondary nodules are found from the sixth month onward. No secondary nodules (i.e., with 'germinal centers') occurred in the material studied. Lymphocytes infiltrate the epithelium of the early, solid ingrowths and are commonly seen in the walls and lumina of all epithelial vesicles. Migration of lymphocytes through crypts other than vesicles is less noticeable.

PERITONSILLAR MUCOUS GLANDS AND DUCTS

The ducts draining the peritonsillar mucous glands arise from the epithelium in and around the tonsillar fossa during the later part of the third month. Since this is before the crypt system has attained any prominence it explains why ducts are rarely found draining into crypts. The following paragraphs will contain summarizations of the data concerning glands and ducts in the various models.

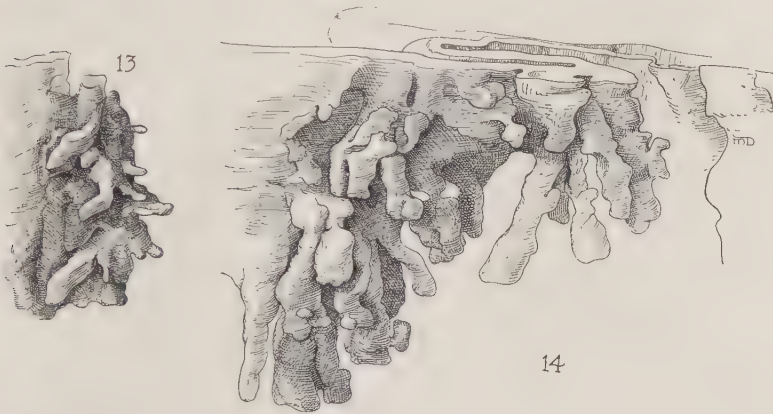


Fig. 13 Drawing of the crypt system in the left tonsil of the 3.5-month fetus ($\times 20$). Original reconstruction, $\times 100$. Supratonsillar fossa (at top center on drawing) presents a long, curved, tubular ingrowth. Mucous glands omitted.

Fig. 14 Drawing of the crypt system in the right tonsil of the 4.2-month fetus ($\times 20$). Original reconstruction, $\times 100$. Compare the complexity of the crypt systems at 3.5 and 4.2 months. The drawing includes a section through a superior part of the tonsillar fossa to demonstrate the relationship of the crypts to its lateral wall. Note the numerous cylindrical crypts; also the crypts with narrow, constricted necks (vesicles). Supratonsillar fossa and mucous glands are not shown.

In the 3.5-month model (fig. 13) there are thirteen ducts; eleven open on the epithelial surface anterior to the tonsillar fossa and two empty into the supratonsillar fossa. Gland masses are not sufficiently developed at this stage to be modelled with ease.

The 4.2-month model (fig. 14) has ten ducts; nine of these open along the anterior margin of the tonsillar fossa and one

opens into the supra-tonsillar fossa. No gland masses were modelled.

The 6.5-month model (fig. 15) has seven gland masses located along the anterior border of the tonsil from the



Fig. 15 Drawing of the crypt system in the right tonsil of the 6.5-month fetus ($\times 18$). Original reconstruction, $\times 75$. Note the presence of large, flat, plate-like invaginations (straight or curved). Observe also the numerous vesicles with narrow necks. A well-developed supratonsillar fossa appears in the upper quarter of the drawing. Black masses represent peritonsillar mucous glands located mostly along the anterior and superior aspect of the tonsillar fossa.

superior to the inferior pole. These glands are drained by thirteen ducts; eleven of these open along the anterior margin of the tonsillar fossa, while the remaining two open on the epithelial margin of the inferior pole.



Fig. 16 Drawing of the crypt system in the right tonsil of a term specimen ($\times 18$). Original reconstruction, $\times 75$. The straight or curved, plate-like type of crypt predominates both in number and size. Vesicles are more numerous than in the 6.5-month specimen. The supratonsillar fossa subdivides into two parts which can be seen in front of the most superior gland mass. Most of the glands are situated along the anterior border of the tonsil and near the tonsillar poles. Another peritonsillar gland mass can be seen posterior to the superior pole.

The term model shown in figure 16 has eight gland masses arranged along the anterior margin and overlapping the two poles. Twelve ducts pass from these gland masses to open on the epithelial margin surrounding the tonsillar fossa.



Fig. 17 Drawing of the crypt system in the right tonsil of a 1-month postnatal specimen ($\times 18$). Original reconstruction, $\times 75$. Observe the simplicity of the crypt system as compared to the term stage. This simplicity is accounted for by the degeneration of numerous vesicles. The supratonsillar fossa is poorly developed in this specimen. Mucous glands omitted.

Both prehyaline and hyaline cartilage were commonly found associated with the glands, located between them and the lymphoid tissue. According to Weller ('23) cartilage between the lymphoid tissue and the mucous glands is common in the tonsil of the adult. The finding of cartilage in this location

in prenatal stages lends proof to Weller's contention that cartilage in this situation is within the range of anatomic variation and has no pathologic significance. It is reasonable to assume that the cartilage is derived from branchial arch mesenchyme.

BLOOD VESSELS

In the earlier stages, before the crypt system has attained much prominence, the blood vessels, with the exception of those supplying the tonsillar plicae, have no constant recognizable arrangement. The future septal vessels can be seen in the sixth month passing between the larger, permanent, plate-like crypts toward the fossal epithelium. Collaterals arise from the septal arteries, pass toward the crypts, give off vascular loops and finally terminate in a subepithelial plexus.

DISCUSSION

Hammar's ('03) description of the developing crypt system is far more complete than that of his predecessors and it must be reviewed in some detail in connection with the present results. Hammar first considered the fate of the second pouch and the development of the tonsillar fossae and plicae. In a 70-mm. (C R) fetus, he then described small, local thickenings on the wall of the tonsillar fossa, and especially on its superior part. Next in his series is the tonsil of a 110-mm. (C R) fetus whose crypts and fossa are quite similar to those in my 100 mm. specimen. Hammar describes this tonsil as having fine, solid, string-like, epithelial proliferations. The three superior ingrowths are larger and have swollen ends—an indication that this 110-mm. specimen is slightly more advanced than my 100-mm. stage which lacks these bulb-like endings. The lower two ingrowths are smaller and pointed. Hammar's 145-mm. specimen is described as having numerous finger-like processes, some long and some short. On the ends of many of these processes are epithelial vesicles containing epithelial pearls. Bud-like, pedunculated branches were also

present and eight to ten comparatively large portions of the crypt system had been isolated (i.e., 'cut-off' from the crypt system). By comparison, the 130-mm. specimen of the present author contains no completely cut off epithelial balls or masses of epithelium. On the other hand, Hammar's illustration of the 145-mm. stage does not show the long, cylindrical type of crypt so characteristic of my 130-mm. specimen. Hammar's 190-mm. specimen possessed many finger-like and club-like ingrowths, most of which contained horny epithelial plugs. Some epithelial vesicles were detached, or were in the process of being cut off. The cut-off portions of the crypt system are not so numerous as in the previous stage. Hammar's models of the crypt systems in the 235-mm., 260-mm. and term-specimens show a gradual increase in the size and number of crypts. His illustrations likewise indicate that the plate-like type of crypt is predominant in size and number in the later prenatal stages. The occurrence of isolated epithelial balls is described in all these stages; five epithelial balls in the term specimen ranged between 0.4 to 0.9 mm. in diameter. Some of the crypts in the term tonsil are mentioned as having one or two branches, others none. In general all of Hammar's observations are in essential agreement with the findings of the present author. However, he omitted the important, early, postnatal stages which reveal fully the partial destruction of the prenatal crypt system and the simplicity of the crypt system which remains.

The crypts of the present series were counted not for the purpose of establishing numerical values, but to indicate roughly the growth tendencies and also the relationship of vesicles in the developing crypt system. These vesicles have an interesting history. In 1888 Retterer concluded that the lymphocytes in the palatine tonsil had an epithelial origin. Nine years later he stated that the secondary follicle arose in toto from the epithelial sprouts. Hammar's ('03) models showed that epithelial balls were cut off during fetal life. His slides also gave evidence that these balls underwent progressive lymphocytic invasion, and so became harder to distinguish

from secondary follicles as the epithelium became more obscure. Hammar pointed out that the nuclei of epithelial cells were large and pale in contrast to the nuclei of lymphocytes. In addition, the external (basal) border of the epithelial structure remained demonstrable and transitional stages between the two types of cells were lacking. In a later paragraph Hammar concluded that these epithelial balls may in part atrophy and disappear under the influence of lymphocytic invasion, and in part be transformed into epithelial vesicles filled with cellular debris. This statement implies that the epithelial balls mentioned first were solid and that these were the ones confused by Retterer with secondary follicles.

The present investigation has given somewhat different results from those of Hammar. Vesicle formation always preceded complete degeneration of the epithelium: that is, no solid epithelial balls were found to atrophy and disappear. Also, the vesicles, even though showing progressive degeneration of their epithelium, tended to persist until after term. In the series examined, solid epithelial balls could not be found entirely detached, although vesicles were found cut off or with doubtful connections in the prenatal stages. However, the pale areas left by the degenerating vesicles might on superficial examination be easily mistaken for secondary follicles of the lymphoid tissue. A small, round or oval area is more easily mistaken for a secondary follicle than an area that branches (crypts of second and third order associated with the degenerating vesicle). In most instances the chief differentiating feature, as already mentioned, is the finding of scattered remnants of flattened, infiltrated epithelium around the margin of the clear area.

Foerster ('23) also investigated the epithelial vesicles in the study of human postnatal tonsils of the first year. He advanced several interpretations that might theoretically account for their presence. First, that they are cysts formed in fetal life, no inflammatory change being involved. Second, cysts formed in fetal life, but with secondary, inflammatory involvement due to adjacent disease processes. Third, cysts

formed through the influence of chronic disease in postnatal life. Fourth, cysts resulting from acute inflammation. Rejecting these possibilities, Foerster finally concluded that the cysts are a product of an atypical developmental phenomenon during fetal life and later may become involved in an inflammatory change. He noticed an increase in the degeneration of the epithelium of the cysts in the inflamed postnatal tonsils, as well as an increase in the number of lymphocytes migrating through the epithelium. This suggests that the inflammatory process may hasten the degeneration of vesicles.

Foerster noted further that the small cysts had a striking similarity to the Hassal's corpuscles of the thymus and cites Mollier ('13) as subscribing to the same opinion. But Hammar ('24), Dearth ('28), and Kingsbury ('28) have shown that the Hassal's corpuscle consists of hypertrophic, degenerated reticular cells. These structures increase rapidly from the second intrauterine month throughout the remainder of fetal life, and then develop more slowly until the period of age involution. Referring now to the tonsil, one finds that the epithelial plug of a small cyst is formed by a degeneration of the central cells and that this plug is destroyed along with the general degeneration of the vesicle. On the basis of this comparison, no more than a superficial similarity between a Hassal's corpuscle and a small cyst is warranted.

Very little is known of the crypt system in lower mammals. Besides the work of Kingsbury and Rogers ('27) on the tonsil of the calf, of Levin ('30) on the pig, and of Ramsay ('35) on the cat, the comparative studies of Hammar ('03), Hett and Butterfield ('10) and Ellenberger and Illing ('11) on various mammalian tonsils serve as the best indication of the nature of the crypt system in lower animals. 'Cutting-off' and degeneration of portions of the epithelium in other mammals has not been reported.

It is hoped that certain investigations, to which the present report is an introduction, will serve as a basis for a better interpretation of the crypt system in childhood and adult life. The crypt system of the palatine tonsil after birth has

also been studied by means of reconstruction methods. Representative stages ranging from a well-developed 3-year-old specimen to an atrophic 65-year-old specimen have been modelled, and the crypt system carefully studied. Those who are interested in following the remaining history of the crypt system are referred to an article on "Prenatal and postnatal development and form of crypts of human palatine tonsil" by Minear, Arey and Milton ('37).

CONCLUSIONS

1. The formation of crypts in the human tonsil dates from the middle of the third lunar month, but the crypt system attains little prominence until the fourth month.

2. Most of the early crypt system consists of cylinders (circular or ovoid in transverse section) which tend to undergo constriction and vesicle formation.

3. In the formation of vesicles, a lumen first occurs in the distal, expanded portion of a solid epithelial ingrowth. Proximal and distal lumen formation, which proceed separately or simultaneously, are found in most crypts.

4. Although most of the vesicles undergo 'cutting-off' and degeneration, a few establish wide communication with the permanent crypt system and are thought to persist into adult life.

5. Vesicles are mostly formed during the early development of the tonsil, but their number increases up to term.

6. A combination of retarded growth to form a neck, followed by degeneration of this neck, is responsible for the 'cutting-off' of a vesicle.

7. The area left by the degenerating vesicle is filled-in, first by lymphocytes and histiocytes and later by the fixed connective tissue elements characterizing lymphoid tissue. Some of these areas might easily be mistaken for secondary nodules.

8. The ingrowths destined to form part of the adult crypt system attain prominence during the sixth lunar month. The larger and more prominent of these are straight or curved, plate-like crypts.

9. The size, shape, position and order of a crypt are determined by the relative growth activity in different parts of the epithelium.

LITERATURE CITED

- DEARTH, O. A. 1928 Late development of the thymus in the cat. Nature and significance of the corpuscles of Hassall and cystic formations. *Am. J. Anat.*, vol. 41, pp. 321-352.
- ELLENBERGER, W., AND G. ILLING 1911 Die Mandeln, etc., in Ellenberger and Baum: *Handb. d. vergl. Anat. d. Haustiere*. Hirschwald, Berlin, Bd. 3, S. 81.
- FOERSTER, A. 1923 Die Entwicklung der Gaumenmandel in ersten Lebensjahr. *Arch. f. path. Anat. u. Physiol.*, Bd. 241, S. 418-427.
- GULLAND, G. L. 1891 The development of adenoid tissue with special reference to the tonsil and thymus. *Rep. Lab. Roy. Coll. Phys.*, Edinburgh, vol. 3, pp. 157-176.
- HAMMAR, J. A. 1903 Studien über die Entwicklung des Vorderdarms und einiger angrenzenden Organe. *Arch. f. mikroskop. Anat.*, Bd. 61, S. 404-458.
- 1924 Über progressive und regressive Formen von Hassallschen Körpern. *Zeitschr. f. Anat. u. Entwickl.*, Bd. 70, S. 466-488.
- HETT, G. S., AND H. B. BUTTERFIELD 1910 The anatomy of the palatine tonsils. *J. Anat. and Physiol.*, vol. 44, pp. 35-54.
- KINGSBURY, B. F. 1928 On the nature and significance of the thymic corpuscles (of Hassall). *Anat. Rec.*, vol. 38, pp. 141-159.
- KINGSBURY, B. F., AND W. F. ROGERS 1927 Development of the palatine tonsil: Calf (*Bos taurus*). *Am. J. Anat.*, vol. 39, pp. 379-435.
- KOLLMAN, J. 1900 Die Entwicklung der lymphknötchen in dem Blindarm und in dem Processus Vermiformis. Die Entwicklung der Tonsillen und die Entwicklung der milz. *Arch. f. Anat. u. Physiol.*, Anat. Abt., Bd. 24, S. 155-186.
- LEVIN, P. M. 1930 The development of the tonsil of the domestic pig. *Anat. Rec.*, vol. 45, pp. 189-201.
- MINEAR, W. L., L. B. AREY AND J. T. MILTON 1937 Prenatal and postnatal development and form of crypts of human palatine tonsil. *Arch. Otolaryn.*, vol. 25, pp. 487-519.
- MINOT, C. S. 1892 *Human Embryology*. Wm. Wood, New York, 815 pp.
- MOLLIER, S. I. 1913 Die lymphoepithelialen Organe. *Sitzungsber. d. Gesellsch. f. morph. u. Physiol. in München*. 29 Reun., S. 14-38.
- RAMSAY, A. J. 1935 The development of the palatine tonsil: (cat). *Am. J. Anat.*, vol. 57, pp. 171-203.
- RETTERER, E. 1888 Origine et evolution des amygdales chez les mammiferes. *J. de l'Anat.*, T. 24, pp. 274-360.
- STÖHR, P. 1891 Die Entwicklung des adenoiden Gewebes der Zungenbalge und der Mandeln des Menschen. *Anat. Anz.*, Bd. 6, S. 454-548.
- WELLER, C. V. 1923 The incidence and histopathology of bone and cartilage in the tonsil. *Ann. Otol. Rhin. and Laryn.*, vol. 32, pp. 687-727.

THE FIRST CONTRACTIONS OF THE HEART IN RAT EMBRYOS

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NINE FIGURES

The earliest contractions of the embryonic hearts which will be described in this communication, were observed in hanging drop cultures of whole rat embryos.

The initiation of contraction has been observed in two other forms, namely, in the chick by Patten and Kramer ('33), Sabin ('20) and Johnstone ('25) and in *Amblystoma* by Copenhaver ('37). According to these authors, the first portion of the heart which becomes active is the ventricle, and the myocardium contracts vigorously for some time before circulation of the blood is established. The development of function in the rat will be seen to follow the same general plan. Certain differences, however, correlated with differences in the morphological development will be described. A comparison of the structure of the rat heart with that of other mammals at the same stage of development suggests that the type of functional activity observed in the rat may be characteristic of mammalian embryos. In order to facilitate the study of the morphology of the heart and associated structures at the stage of beginning contraction, a wax plate reconstruction was prepared of a rat embryo of corresponding age fixed in the uterus.

MATERIAL AND METHODS

The technique for removal of rat embryos and their preparation in hanging drop tissue cultures has been described by Nicholas and Rudnick ('34) and a few modifications

are given by Goss ('35). The medium which gave the most favorable results was composed of 1 part chicken plasma and 4 parts extract of newborn rat hearts in a buffered salt (Tyrode) solution.

The age of the embryos at the time of initiation of contraction was estimated at approximately $9\frac{1}{2}$ days. It is not possible to make an exact determination of the age in days and hours for two reasons. First, variations in the stage of development of embryos in the same litter frequently correspond to as much as 4 to 6 hours of normal development. That is, in the same litter embryos are obtained which have no recognizable heart primordium along with embryos in which the heart has developed into a saccular organ that is contracting vigorously. Second, a similar or greater variation in stage of development is found between embryos of different litters although they are of the same age as calculated from the time of copulation. The most accurate designation of age that can be given as a result of these experiments is 9 days and 14 ± 2 hours.

The following procedure, worked out by experience, has been found useful to obtain embryos of the desired age without resorting to the tedious method of observing the copulation time. As a routine, the oestrous cycles of the female rats in the colony were determined by vaginal smear between the hours of 9 and 11 in the morning. When a female was found to be in proestrus or 'stage one' (of Long and Evans, '22), she was placed in a cage with a male. If sperm was recovered in the vaginal smear next morning, it was assumed that copulation had taken place during the early morning hours. (According to Long and Evans, stage one lasts 12 hours and mating takes place in the early hours of stage two). The majority of embryos in a litter removed from the uterus after noon of the tenth day were, in most cases, in the desired stage of development. Embryos obtained before noon were usually too young and those removed after 4 o'clock, too old.

STAGE OF DEVELOPMENT

The developmental age of the embryos can best be determined by a description of the embryo at the time of beginning heart activity. The embryonic vesicle or blastocyst is approximately 2 mm. in length. It corresponds in most respects to stage 16 of Nicholas. Figure 8 is a sketch of a living embryo immediately after removal from the uterus.

The medullary folds and the foregut invagination are the most conspicuous structures. The cephalic portion of the neural folds forms a prominent forebrain which extends

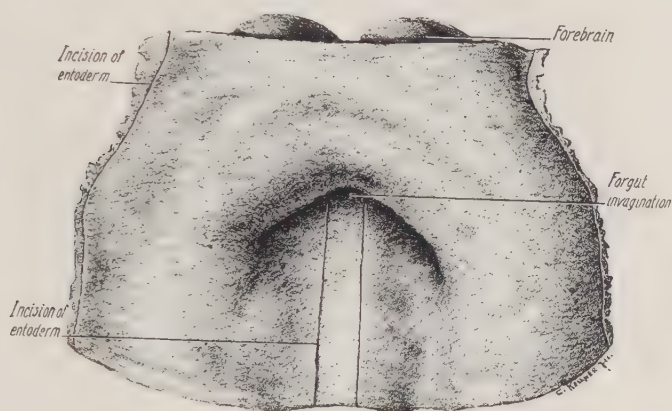


Fig.1 Wax plate reconstruction of cephalic portion of 9-day 16-hour rat embryo fixed in utero. Viewed from ventral surface. $\times 400$ reduced to $\times 100$.

cephalad beyond all other portions of the embryo. The neural groove is deep in the cephalic region but gradually becomes shallower until there is merely a medullary plate at the caudal end of the embryo. The allantois is a blunt projection which lies outside of the amnion, extending into the cavity of the yolk sac. The number of somites varies from 2 to 4. The embryo in figure 1 had 3 somites, that in figure 8 also had 3. The number of somites was found to correspond less closely to the stage of development of the heart and nervous system than the latter did to each other. The wall of the yolk sac contains both solid primitive blood islands and

those with endothelial lined cavities and free blood cells, but the latter have not yet taken on the yellow color of hemoglobin. The pericardial cavity is shaped like the letter U. The curved portion lies close against the lip of the anterior intestinal portal. The arms extend back to the region of the somites where the pericardial cavity is connected with the extra-embryonic coelom. The heart and its associated structures will require a more detailed description.

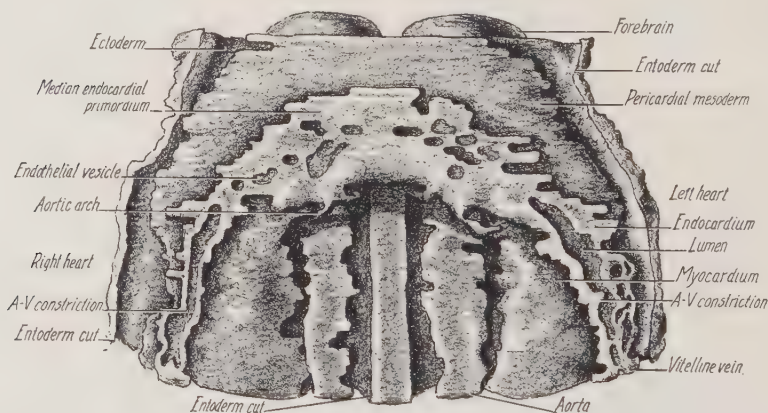


Fig. 2 Same reconstruction as in figure 1 with most of endoderm removed.

DESCRIPTION OF THE HEART

The difficulty of following the delicate endothelial structures in the living embryo was so great that a wax plate reconstruction was prepared of an embryo fixed in the uterus. This particular embryo was selected from a number of fixed specimens because it most closely resembled the living embryos in development. Figures 1 to 5 are drawings of the reconstruction.

The endocardium consists of two parts, the lateral primordia which are endothelial tubes and a median portion which has no lumen. Both parts are combined into a continuous network or sheet of cells (fig. 2) which is everywhere in contact with splanchnic mesoderm. In the median portion, small scattered vesicles (fig. 2) represent the earliest develop-

ment of a lumen. In this condition the tissue is more accurately termed a primordium of endocardium than primitive endocardium. It is the angioblast of many authors. Except for the scattered vesicles, the median portion is a lamina composed of a single layer of flattened cells. It is definitely separated from the entoderm throughout and from the

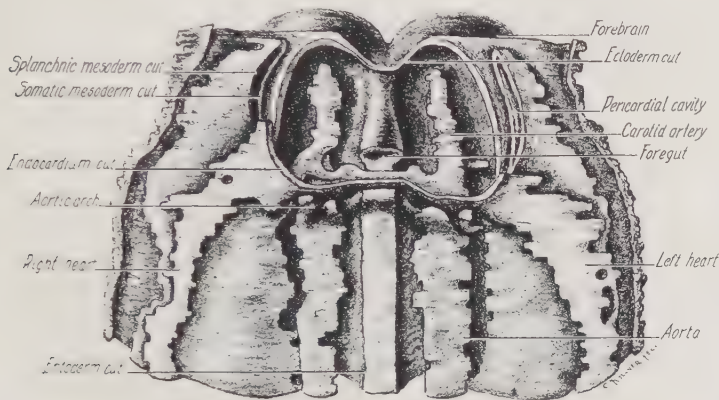


Fig. 3 Same as figure 2 with windows cut through endocardium and pericardial mesoderm in order to show internal structures.

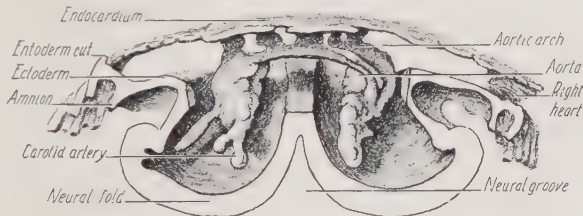


Fig. 4 Same reconstruction as in figure 3. The cephalic portion of the neural folds and the pericardial mesoderm have been removed. Viewed from the cephalic end.

splanchnic mesoderm except at its cephalic border. Strands of cells from the lamina pass around the foregut on both sides to become continuous with the primitive dorsal aortae. They are the primordia of the first aortic arches and that portion of the lamina which lies between them will be incorporated into the aortic sac (figs. 2, 3 and 4). The aortae are endothelial tubes, patent from the arch primordia to the

region caudal to the somites. They are continued into the cephalic mesenchyme as the primitive carotid arteries (fig. 5).

The endothelial tubes of the lateral heart primordia cross the lateral plates of splanchnic mesoderm diagonally. They form a shallow arc with its convexity away from the midline. At the outer edges of the lateral cardiac plates, the tubes merge with the primordia of the veins which may contain scattered vesicles but do not have a continuous lumen. A short distance from this junction with the veins, the lumen of

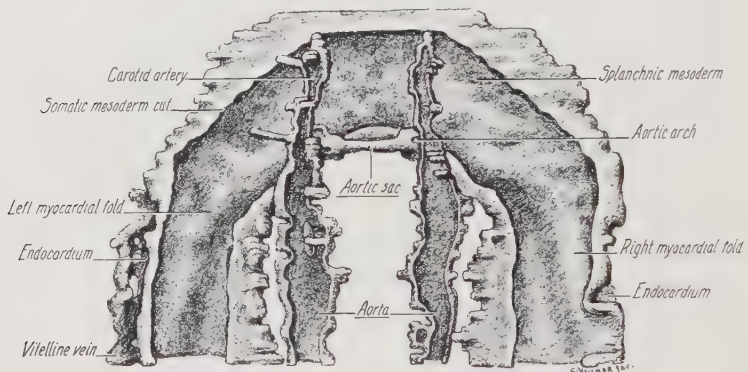


Fig. 5 Pericardial mesoderm and endocardium of same reconstruction as in figure 2 viewed from the dorsal surface. The somatic layer of mesoderm has been cut away in order to show the myocardial folds of the splanchnic layer. The arteries are cut open to show the extent of their lumen.

each tube is constricted by an inward projection of endocardium which resembles an imperfect valve. This constriction is remarkably constant in its form and location. It marks the separation of the future atrium and ventricle, often called the atrioventricular canal, but at this stage it is a sharp constriction rather than a canal. It is the most important landmark for determining the area in which to observe the heart for its first contractions. The portion of the tube between the veins and the constriction was identified as the atrium by its activity in older embryos.

The myocardium corresponds to the endocardium in having a single median portion and two lateral portions. It is formed by an infolding of the splanchnic mesoderm (fig. 5).

The infolding of the median portion is scarcely distinguishable at this stage. The folds of each lateral plate form an incomplete tube which partially surrounds the endocardial tube. Figure 6 is a section cut through both hearts of the embryo used for reconstruction. It illustrates the arrangement of cells and relative size of the parts. Occasional strands of protoplasm cross the space between the endocardium and myocardium, but they are much less frequently seen in the living embryo than one would expect from the appearance of fixed specimens. There is a slight thickening and constriction of the myocardium at the point where the endocardial projections mark the A-V junction.

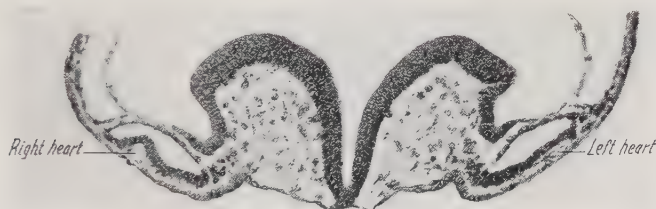


Fig. 6 Photomicrograph of section of 9-day 16-hour rat embryo, through lateral heart primordia. Same embryo used for reconstruction of figures 1 to 5. $\times 130$.

The heart is bilaterally symmetrical at this stage. The two slides are mirror images of each other, but certain slight differences may be seen, particularly in the living embryo. They foreshadow greater differences which become manifest in later development. The right endocardial tube is more sharply defined and its diameter more uniform than the left. The central portion of the left tube is slightly dilated or sacular. The lumen of the right often extends farther into the median lamina. The right side usually gives the impression of being better developed morphologically than the left.

INITIATION OF CONTRACTION

Preliminary experiments were performed on a number of litters in order to discover at what age and in what area the

first contractions occurred. All the embryos obtained from each litter which were suitable for study (usually from four to six) were examined for short periods in rotation. A record was kept of the age, length of time in culture, and the presence and location of cardiac contractions. In the embryos which developed most normally, the portion of the heart first seen in activity was the left outer or lateral fold of the ventricular myocardium near the A-V constriction at a stage of development corresponding to figure 8. Independent activity began in the right heart approximately 2 hours later than in the left.

In some of the cultures, although the embryos appeared uninjured, the myocardium of but one side contracted. In others both sides were active, but they failed to unite subsequently into a single heart. This effect was interpreted as the result of uneven mechanical pressure, because it had been found in earlier experiments (Goss, '35) that changes of this kind could alter the course of development of the heart. These embryos were not accepted as the primary source of data for determining the origin of contraction, but they provided valuable confirmatory evidence. For example, in all the cases in which the left heart contracted at any time, twenty-two in number, it was the first to be found in action. The right showed activity first in but two cases and in both of them the left heart was never seen in contraction.

After the correct stage of development and the first active portion of the heart had been determined, it was possible to set up a somewhat younger embryo (fig. 7) for continuous observation. The culture was left in the incubator for 2 hours to allow the plasma to clot and the embryo to become adjusted to its new environment. It was then placed on the stage of a microscope enclosed in an incubator at 38°C. and observed with a 40 × objective and 15 × oculars. No motion of the myocardial cells could be detected. Cinematographs were taken of the heart region and observation with the microscope resumed.

At the end of 30 minutes of continuous observation, during which no motion could be detected, a slight twitching of one or two cells was seen in the lateral wall of the ventricular portion of the left heart near the A-V junction. The excursion of these first contractions was so slight that it was difficult to be certain that they were all observed, but those which were recognized came with surprising regularity. In 10 minutes the excursion had increased so that counting was feasible. The rhythm was then regular and contractions occurred at the rate of 37 per minute. After records were made of the left heart,

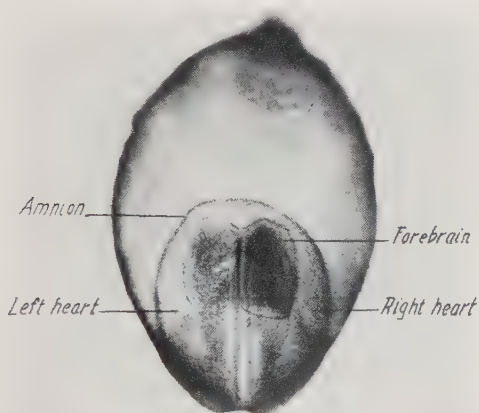


Fig. 7 Living rat embryo approximately 9 days 12 hours. Photograph. $\times 25$.

the right was examined carefully but no motion could be seen. Continuous observation was discontinued, but the right heart was examined at intervals. No activity was seen until 2 hours had elapsed. Its first contractions were regular but less frequent, by 2 or 3 contractions, than those of the left. During these 2 hours the rate of the left side had increased gradually to 43 per minute. The difference between this increased rate and the beginning rate of the right side was sufficiently great to make the independence of rhythm on the two sides quite obvious.

Gradually, all the ventricular myocardium of both sides became involved in the contraction. The spread of activity was first along the outer or convex wall toward the median line and then across the tube to take in the inner or concave wall. By the time all the myocardium surrounding the endocardial tube was active, a wave or peristaltoid action suggested itself and was strikingly confirmed in slow-motion cinematographs. The wave began in the portion nearest the A-V constriction and travelled toward the median region. The tube on the venous side of the constriction, i.e., the atrium, remained inactive.

Morphological development accompanied the changes in activity. The pericardial cavity increased surprisingly in its extent. The lumen of the endocardial tubes extended farther toward the midline. The myocardial tubes lengthened and increased in diameter. The fold in the median part of the myocardium became as distinct as the lateral folds so that it united the lateral or convex walls of the lateral myocardial tubes into a single saccular ventricle. A similar fold connecting the inner or lesser curvatures could not be distinguished because of its proximity to the foregut portal and because of the openings of the aortic arches. The contraction gradually involved more and more of the median portion until it finally spread across the midline. The independent activity of the right side was then suppressed and the peristaltoid wave travelled from the A-V region of the left side to the A-V region of the right. In one of the preliminary cases, the right and left hearts became synchronized before motion was observed in the median portion of the myocardium.

The early activity was observed in nine embryos. In general, all were similar to the case just described. The rates of the first contractions varied from 34 to 42, at 38°C. Irregularities such as intermittence and temporary cessation of contraction were observed, but a complete irregularity was only observed after the embryos had been in culture for more than 48 hours. The irregularities in fresh cultures could be

explained by environmental changes in all cases. When the heart was beginning its activity it was extremely susceptible to changes in temperature and mechanical agitation, both of which caused temporary cessation of the beat, frequently



Fig. 8 Living rat embryonic vesicle approximately 9 days 14 hours. Drawing from sketches made immediately after removal from uterus.

followed by an abnormally high rate. A variation in temperature of 4 or 5° sometimes changed the rate by eight or ten contractions per minute. When the cultures were undisturbed for a period of 10 minutes at a constant temperature, the rhythm became regular and the rates agreed reasonably well with the rates of other embryos at the same stage of development.

The two lateral hearts were slightly different, both in form and activity. The left side had a more rapid rate than the right except in cases which appeared abnormal. As the left side developed it tended to become saccular and its contractions were more sudden and less sustained than those of the right. The right tended to retain its tubular appearance and

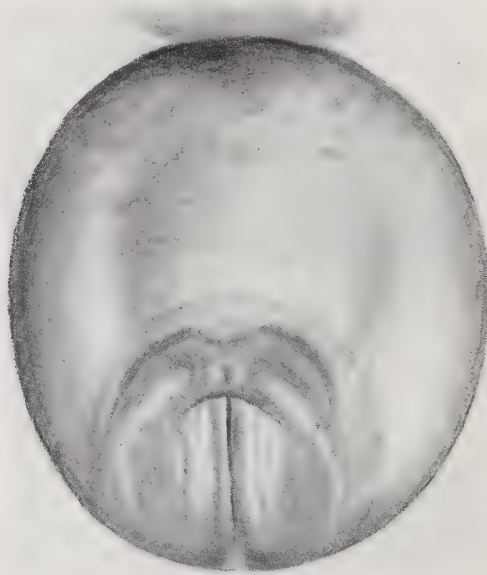


Fig. 9 Rat embryonic vesicle, after 16 hours in hanging drop culture. Lateral hearts were contracting independently. $\times 40$.

the wave of contraction travelled more slowly so that it gave the impression of a more powerful constriction. Of the nine embryos observed, three formed single hearts, six formed double hearts. The failure of the two hearts to join has been explained (Goss, '35) as due to pressure against the cover glass. When the two hearts remained separated, the differences between them became exaggerated as the cultures developed.

In all cases in which the single median ventricle was formed, the resulting structure was saccular, rather than tubular. Al-

though a clearly tubular form, such as occurs in the chick, was not to be expected in a mammal, the saccular form in these embryos may have been exaggerated by the conditions in culture. Some distortion was always caused by fluid changes in the cultures. Within 2 or 3 minutes after removal from the uterus, there was a loss of fluid from all the cavities in the embryonic vesicle. It appeared shrunken and slightly more opaque. After 2 hours in culture, the volume of the vesicle was restored but the resulting form was abnormally spherical. The change in shape of the vesicle becomes clear in a comparison of figure 8 which is a drawing based upon sketches made immediately after removal from the uterus and figure 9 which is a drawing after 16 hours in culture.

The formation of a continuous endothelial lumen appeared to be a very important factor. In normal development, the lumen of the aorta extends through the first aortic arches to that of the heart shortly before the ventricle becomes a single structure. The lumen was not established in most of the cultured embryos. In the few which formed a lumen, development of the heart greatly surpassed that of the other embryos. In one case of failure of the right and left hearts to unite, each heart became connected with the aorta of its own side. Development continued until it appeared that the hearts would have forced the blood into circulation if the blood vessels had formed their proper connections.

Circulation was not observed in any of these embryos. The connections between arteries and veins were not patent. The examination of older embryos soon after removal from the uterus indicated that in normal development the circulation does not begin until the embryo has developed approximately 12 hours beyond the stage of first contraction.

No cytological changes in the cells of the myocardium could be distinguished as a result of their earliest contraction. In the living hearts, the shape of the cells remained the same and no rearrangement of mitochondria or formation of fibrillae was observed. Since a knowledge of changes accompanying

the initiation of activity in these cells is very important for the cytological background of muscular contraction, a detailed study of fixed material has been undertaken and will be reported later.

DISCUSSION

Our observations with the rat confirm those with the chick by Sabin ('20), Johnstone ('25) and Patten ('33) and those with *Amblystoma* by Copenhaver ('37), that the first portion of the heart which becomes active is the primitive ventricle. Sabin states that the first contractions have a regular rhythm and Johnstone implies the same when he speaks of the rhythmic activity, but Patten reports that they do not appear regular when recorded by a method more precise than observation through the microscope. The author used Patten's method of superimposing enlarged cinematographs of the heart before contraction was observed, but was unable to distinguish earlier movements. It is possible that the 16-mm. film used by the author gave less accurate results than the larger film used by Patten. In *Amblystoma*, Copenhaver (personal communication) found that the first observed contractions had a regular rhythm in the majority of cases. Irregularity and intermittence were recorded in some cases and he raises the question as to whether these embryos were showing normal activity. Our observations with the rat, as far as they have gone, agree with those of Sabin and Copenhaver that the earliest contractions tend to have a regular rhythm.

In a brief report of some of the earlier preliminary experiments (Goss, '37), the first contractions were given a rate more rapid than that contained in this communication. Our more recent observations were made on a larger number of embryos and the experimental conditions were more carefully controlled during continuous observation. We conclude, therefore, that the preliminary rate was too high, probably because it was based in part on embryos which had passed the stage of earliest contraction.

Sabin, Johnstone and Patten all found that the right side of the heart initiated contraction in the chick. Copenhaver observed the first contractions on one side in seven out of twenty-four *Amblystoma* embryos. In six of these the first motion was limited to the left side and in one it was definitely limited to the right side. Apparently the first contractions in *Amblystoma* can appear on either side and very quickly extend over both sides. In the rat, we found that the left side became active first except in those cases in which there was reason to believe that this side had been retarded or suppressed.

Cardiac activity is developed more precociously in the rat than in the chick and *Amblystoma*. In both the latter forms, the first contractions were observed after the lateral primordia had fused into a single tubular heart, whereas in the rat they occurred while the lateral hearts were still separated. In the rat, moreover, the first contractions appear in the 3-somite stage, while in the chick they appear in the 10-somite stage and in *Amblystoma* between stages 33 and 34 (16 to 18 somites). Not only does the activity begin before the ventricle is a single organ, but the two parts develop independently and each has its own power of rhythmical contraction. It is not possible to say with certainty that this precocity occurs in normal development in utero because the conditions in vitro may introduce abnormalities. On the other hand, in embryos examined soon after removal from the uterus, the independent activity of the two sides was observed if the middle cardiac plate had not developed, and the whole heart was synchronized if the median portion had united the lateral portions into a saccular ventricle.

Sabin and Johnstone observed a gradual spread of activity over the ventricle from the localized region of initiation near the veins to the origin of the arteries. It first extended along the right border or greater curvature and then along the lesser curvature. Patten also observed that the earliest activity was on one side, but he found that the spread was irregular. In fourteen of the twenty-four *Amblystoma*

embryos studied, Copenhaver found that the first observed contractions involved most of the anterior two-thirds of the heart (ventricle and bulbus). Although he observed the first contractions one side in seven cases, they were not always on the same side and apparently spread quickly over both sides. The activity in the rat heart extended from a small localized area along the lateral border or greater curvature and then across the tube to the lesser curvature. Finally all the myocardium surrounding the endocardial lumen was involved. In general this was a spread from the region near the veins to that near the arteries.

After the entire primitive ventricle had become active in the chick, Patten observed a 'peristaltoid' wave in the contraction. It travelled from the venous to the arterial end. The same kind of wave was observed in the rat. While the lateral hearts were still independent, the wave travelled from venous to arterial ends as in the chick. After the median portion of the myocardium had become active, the wave travelled from side to side, i.e., from vein to vein, rather than from posterior to anterior or from veins to arteries. This difference seems to be due to the morphology of the heart after a single saccular ventricle has been formed. There is a greater and lesser curvature, as in the chick, but they extend from the A-V junction of one side to the A-V junction of the other side. The aortae open near the lesser curvature, leaving the greater curvature as the expanded and more motile portion.

Yoshinaga ('21) in his description of the development of the guinea pig heart writes:

the cranial prolongation of the lateral myocardial tubes has not been brought about by the direct extension of the first part of the myocardial tubes, but by the continuous progressive differentiation into the craniomedian limb of the pericardial cavity. Therefore, the confluence of the myocardial tubes into a single myocardial cavity is not accomplished by the actual fusion of the bilateral myocardial tubes, followed by absorption of the septal walls.

In the rat a similar condition was found. Instead of a fusion of the two lateral myocardial tubes, there was a more or less independent growth of the median portion which eventually united them into a saccular organ.

The stage at which the hearts of other mammals begin to contract cannot be determined from morphological studies alone. The author has found several published accounts, however, which include descriptions of a stage which is sufficiently similar to that of the rat to warrant a brief discussion of the probable time of earliest cardiac activity in other mammals. Hensen (1876) described the two lateral primordia in the rabbit and guinea pig and commented on their differences from the early chick heart. Although the rabbit embryo depicted in his figures 28 and 37 is too young for beginning contraction, it is close to this stage. He traced the formation of the endocardial tubes from scattered cells of the 'vessel layer' shown in his figure 37. The myocardium he derived from the visceral layer of the primitive pericardial cavity.

The rabbit has been illustrated by Bremer ('12) and Rouvière ('04). Bremer's figure 3 is based on a very carefully prepared reconstruction of an 8½-day rabbit with 6 to 7 somites. The condition of the heart and endothelium corresponds closely to that which we have observed in the rat. The pericardium and heart of a 201-hour rabbit in Rouvière's plate 17, figure 1 also seem to be of the same stage.

The heart of the guinea pig embryo in Yoshinaga's ('21) stage III (figs. 12 and 13) has a myocardium which is slightly better developed than that of the rat at the beginning contraction. The general development, however, is the same. The extent of the lumen in the endothelial tubes is similar, and it is worthy of notice that the right side extends farther into the median region than the left, as we have mentioned in the rat. The endocardial primordium or angioblast, according to Yoshinaga, is much less extensive in the median region of the guinea pig embryos, at this stage.

The ferret embryo with 5 somites which Wang ('17) has given in graphic reconstruction in his figure 6a corresponds

in general to the stage we have described in the rat. The continuity of endocardial lumen across the midline is difficult to understand because the lumen is interrupted in the lateral hearts. We are inclined to disagree with Wang's interpretation of the various parts of the vascular system. What he has labelled vitelline vein seems to be the heart tube and the connection between the vitelline vein and aorta which he describes is apparently the aortic arch primordium.

The reconstruction of a cat embryo illustrated in figure 1 by Schulte ('16) has many resemblances to that of the rat which we have given. It is worthy of notice, however, that although this cat embryo had 8 somites, it had no foregut invagination. The author has examined the slides from which the reconstruction was made and has found that the cardiac primordia were further developed cytologically than the reconstruction would indicate. It seems probable that development had gone slightly beyond the stage of beginning contraction.

Parker ('15) gives a graphic reconstruction (plate I, fig. 3) of a marsupial embryo (*Dasyurus viverrinus*, 8.5 mm.) in which the heart probably had reached the stage of first contraction. The foregut and brain are represented, but there are no somites. The endothelial tube of each lateral heart is continuous with that of the aorta of the same side through the first aortic arch. The lumen, however, does not extend across the midline to form a communication between the lateral tubes. The condition of the heart in older marsupial embryos indicates that functional development progresses farther before union into a single ventricle than is the case in the rat.

No description of a human embryo in which the heart corresponds to the beginning of contraction, has been found. Such a stage would be intermediate between embryo 5080 of Davis ('27) and the Ingalls ('20) embryo. In the former the pericardial cavity has formed but there are no myocardial folds. In the latter the folds have been partly united by the median myocardium. The Davis 5080 embryo has 1 somite forming, the Ingalls has 2 somites. There is good reason to believe,

however, that the development of the human heart follows the same plan as that of other mammals and that the desired stage will be found as more human material becomes available.

SUMMARY

The beginning of contractile activity in the hearts of rat embryos was observed in hanging drop cultures of whole embryonic vesicles. The age of the embryos was approximately 9 days 14 hours. The hearts were observed continuously from $\frac{1}{2}$ hour before contraction until activity was well established.

The first contractions have a regular rhythm and a rate of 37 to 42 per minute. They are confined to a small area, three or four cells, in the lateral ventricular myocardium of the left primitive heart tube. The area is near a constriction which marks the junction of the primitive atrium and ventricle.

The right myocardial tube begins contracting in a similar fashion 2 hours after the left. Its rhythm is regular, but independent of and slower than the left. The contractile activity gradually extends over the ventricular myocardium of each side until all the portion surrounding the endocardial lumen is involved.

The two lateral hearts become united into a single saccular ventricle by the progressive differentiation of the median splanchnic mesoderm. After this median myocardium becomes active, the independent activity of the right heart is suppressed and the left side becomes the pace-maker for the whole ventricle. The rate increases gradually as more myocardium becomes contractile. The atrium remains motionless during this development and there is no circulation of the blood.

The contraction has a wave-like character. In each of the lateral heart tubes before their union, it travels from the venous region, i.e., the A-V constriction, to the arterial region of the aortic arch primordia. In the united ventricle, it travels from the A-V junction of the left side to that of the right.

Significant morphological and functional differences between the two primitive lateral hearts occur.

The embryo at the stage of beginning contraction is described from living material and wax reconstruction and compared with other mammalian embryos.

LITERATURE CITED

- BREMER, J. L. 1912 The development of the aorta and aortic arches in rabbits. *Am. J. Anat.*, vol. 13, pp. 111-128.
- COPENHAVER, W. M. 1937 Correlation in the structural and functional development of the heart as studied by heteroplastic grafting. *Anat. Rec.*, vol. 67 (Suppl. no. 3), p. 12.
- DAVIS, CARL L. 1927 Development of the human heart from its first appearance to the stage found in embryos of 20 paired somites. *Carnegie Inst. Pub.*, no. 380, *Contrib. to Embryol.*, vol. 19, pp. 245-284.
- GOSS, C. M. 1935 Double hearts produced experimentally in rat embryos. *J. Exp. Zool.*, vol. 72, pp. 33-49.
- 1937 Early development of the rat heart in vitro. *Anat. Rec.*, vol. 67 (Suppl. no. 3), p. 20.
- HENSEN, V. 1876 Beobachtungen über die Befruchtung und Entwicklung des Kaninchens und Meerschweinchens. *Zeit. f. Anat. u. Entwickl.*, Bd. 1, S. 213-273 and 353-423, *Heart*, S. 367-369.
- INGALLS, N. W. 1920 A human embryo at the beginning of segmentation, with special reference to the vascular system. *Carnegie Inst. Pub.*, no. 274, *Contrib. Embryol.*, vol. 11, pp. 61-90.
- JOHNSTONE, P. N. 1925 Studies on the physiological anatomy of the embryonic heart. *Johns Hopkins Hosp. Bull.*, vol. 36, pp. 299-311.
- LONG, J. A., AND H. M. EVANS 1922 The oestrous cycle in the rat and its associated phenomena. *Mem. Univ. California*, vol. 6, pp. 1-148.
- NICHOLAS, J. S., AND D. RUDNICK 1934 The development of rat embryos in tissue cultures. *Proc. Nat. Acad. Sci.*, vol. 20, pp. 656-658.
- PARKER, K. M. 1915 The early development of the heart and anterior vessels in Marsupials, with special reference to *Perameles*. *Proc. Zool. Soc.*, London, pp. 459-499.
- PATTEN, B. M., AND T. C. KRAMER 1933 The initiation of contraction in the embryonic chick heart. *Am. J. Anat.*, vol. 53, pp. 349-375.
- ROUVIÈRE, H. 1904 Études sur le développement du péricarde chez le lapin. *J. de l'anat. et de la physiol.*, T. 40, pp. 610-633.
- SABIN, F. R. 1920 Studies on the origin of blood-vessels and of red blood-corpuscles as seen in the living blastoderm of chicks during the second day of incubation. *Carnegie Inst. Pub.*, no. 272, *Contrib. Embryol.*, vol. 9, pp. 213-262.
- SCHULTE, H. v.W. 1916 The fusion of the cardiac analages and the formation of the cardiac loop in the cat (*Felis domestica*). *Am. J. Anat.*, vol. 20, pp. 45-72.
- WANG, C. C. 1917 Earliest stages of development of the blood vessels and the heart in ferret embryos. *J. Anat.*, vol. 52, pp. 107-185.
- YOSHINAGA, T. 1921 A contribution to the early development of the heart in mammalia, with special reference to the guinea pig. *Anat. Rec.*, vol. 21, pp. 239-308.

A CASE OF LEFT SUPERIOR VENA CAVA WITHOUT A CORRESPONDING VESSEL ON THE RIGHT SIDE

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THREE FIGURES

Although more than 175 cases (compare McCotter, '16, and Beattie, '31) of left superior vena have been reported, in only about one-tenth of these has the right superior vena cava been absent. Because of the rarity of the latter condition the following report of a new case seems justified. Two previous cases of double superior vena cava have been reported from this laboratory (Huffmire and Bower, '19, and Keyes and Keyes, '25).

DESCRIPTION OF CASE

The case to be described is that of a heart obtained during dissection of the body of a white man, aged 67. The cause of death was recorded as myocarditis.

The heart appears normal in size. Its widest transverse diameter is 11 cm. From the uppermost limit of the right auricle to the apex, the heart measures 14 cm. Externally the several heart chambers appear normal in size and contour. The aorta arises in the usual manner and the arch gives off its three main branches in the usual sequence. There is no evidence of a vena cava on the right side.

The right innominate vein is formed by the union of four veins, the two largest of which are the right subclavian and the right internal jugular. The right innominate vein is 75 mm. in length and passes downward and to the left. During its course it receives two small veins. One enters from

above about 10 mm. from the origin of the innominate and the other enters from below about 30 mm. beyond its origin. It was not determined whether this latter vein is the right internal mammary or a possible right superior intercostal vein.

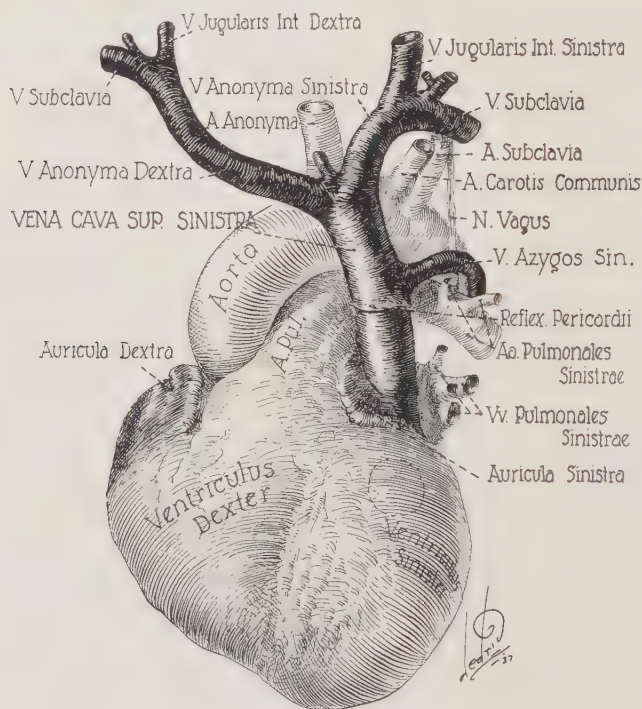


Fig. 1 Heart and great vessels, viewed from the front. $\times \frac{2}{3}$. In this and the succeeding figures the labels and abbreviations follow the Latin form of the Basle Nomina Anatomica.

The left innominate vein is formed by the union of four veins, principal among which are the left subclavian and the left internal jugular. The latter vein measures 11 mm. in diameter while the right internal jugular has a diameter of 7 mm. The left innominate vein is 25 mm. in length and courses downward and only slightly toward the right. The two innominate veins and the inferior thyroid vein join in front of the arch of the aorta to form the left superior vena cava.

The left superior vena cava, of the size of the superior vena cava usually found on the right side, courses downward in front and to the left of the arch of the aorta, to the left of the root of the pulmonary artery and in front of the left pulmonary artery and veins and the left bronchus, and behind the auricular appendage of the left atrium (figs. 1 and 2). In the latter part of its course it occupies the usual position of the vestigial fold, or ligament of the left superior vena cava, and the oblique vein of the left atrium (Marshall).

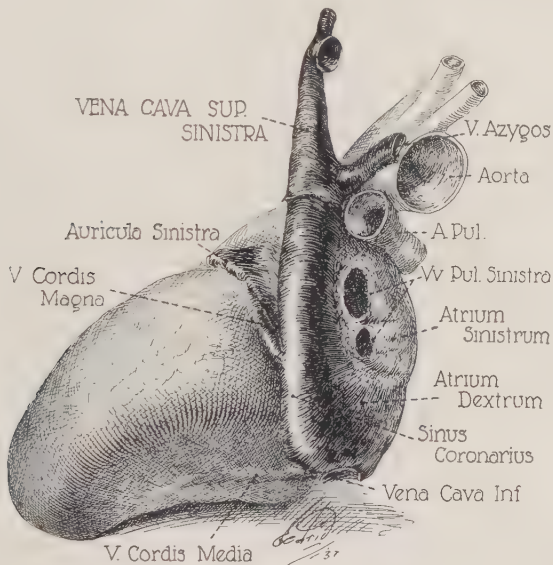


Fig. 2 Heart and great vessels, viewed from the left. $\times \frac{2}{3}$.

After receiving the great cardiac vein, the left superior vena cava continues as a much dilated coronary sinus (fig. 2) which measures 35 mm. in greatest width as it lies in the coronary sulcus.

The enlarged coronary sinus opens into the right atrium at its left and posteriorly. Its orifice is nearly circular and measures approximately 25 mm. in diameter (fig. 3). There is no evidence of a valve (thebesian). The lower margin of the orifice is on a level with the opening of the inferior vena

cava, which likewise fails to show any evidence of a valve (eustachian). The fossa ovalis is pushed upward and forward by the large orifice of the coronary sinus. The fossa is narrower and more elongated than usual and exhibits only a poorly developed limbus. The middle cardiac vein does not empty into the coronary sinus. Its separate opening is located below and in front of the orifice of the sinus (fig. 3).

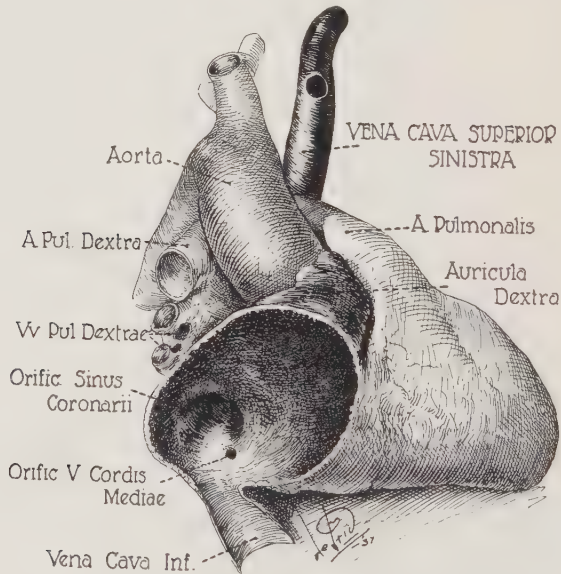


Fig. 3 Heart and great vessels, viewed from the right. A portion of the wall of the right atrium has been removed, displaying the interior of this chamber. $\times \frac{2}{3}$.

About 35 mm. from its origin the superior vena cava receives a large left azygos vein (fig. 1). This vein was on the left side of the mediastinum and for a considerable part of its course was moulded against the left side of the descending thoracic aorta. It arches above the root of the left lung and passes forward and to the right to join the superior vena cava. This left azygos vein receives a large vein from the right side, thus presenting a reversal of the usual condition of the azygos system.

The heart shows some interesting pathological changes. When the left ventricle was incised along the left margin of the heart, the site of an old coronary vessel occlusion was revealed. Here there is an aneurism formation of the wall of the ventricle with fibrosis. There is, however, only slight bulging. The location and approximate extent of the involved area is indicated by the dotted circle shown in figure 1. The thinnest part of the wall measures about 0.5 mm. in thickness. The fibrosed wall along the left margin is about 5 mm. thick in contrast to the left ventricular wall in its most anterior part which is 12 mm. thick. The anterior papillary muscle of the left ventricle is considerably fibrosed.

All six cusps of the aortic and pulmonary semilunar valves show congenital fenestration. There are two right coronary arteries arising side by side. One is approximately three times the size of the other.

PREVIOUS CASES

In the accompanying table are arranged, in chronological order, the known cases of a left superior vena cava with absence of the right vena cava. Only such cases are included as

TABLE 1

Previous cases of superior vena cava on the left side, without corresponding vessel on the right side

Halbertsma	1862	Dietrich	1913
Schmidt	1871	Schütz (2 cases)	1914
Greenfield	1876	Nützel	1914
Gruber	1880	Smith	1916
Weigert	1881	Mönckeberg	1921
Bedart	1892	Benda	1924
Boyd	1893	de la Villa	1926
Mäusert	1899	Halpert and Coman	1930
Gorzuloff	1910	Beattie	1931

are obviously the end result of a reversal of the usual developmental conditions involving the anterior cardinal and common cardinal veins. Cases of situs inversus viscerum have been excluded. We have also omitted the case of Cheselden (1713) cited as the earliest example of this condition, and the case

of Charles (1889). The complete text of Cheselden's description is as follows: "A Heart, with *Vena Azygos* inserted into the right Auricle; and the Descending *Cava* coming round the Basis of the Heart, above the *Aorta* and Pulmonary Vessels, to enter the Auricle at the lower part with the Ascending *Cava*."

Included in the table is the case of Schmidt (1871) which apparently has escaped the notice of most writers on left superior vena cava. This description, in Norwegian, is presented in detail with two illustrations. The heart described by Schmidt was that of a young man, recently discharged from the army, who had presented no signs of circulatory disturbance during his last illness. Abstracted, Schmidt's principal findings are: There was no right superior vena cava. The right innominate vein runs in front of the branches of the aorta, passing downward and to the left to meet the short, more vertically-descending left innominate vein. The left superior vena cava, making a curve which is convex to the left, passes in front of the left pulmonary artery and the left pulmonary veins, and behind the root of the left auricular appendage. It continues into the enormously dilated coronary sinus which is located, as usual, in the posterior part of the coronary sulcus. The sinus opens into the right atrium in front of the orifice of the inferior vena cava and slightly below it. Only traces of a valve for the coronary sinus or for the inferior vena cava could be found.

DISCUSSION

Although the cause of death in our case is given as myocarditis, and the specimen shows evidence of a small healed coronary occlusion, no evidence exists that the reversal of the superior caval and the azygos systems was a chief or even contributory factor.

In our specimen the left internal jugular vein is considerably larger than the right. This may indicate that the superior sagittal sinus of the dura passed to the left instead of to the right. Unfortunately no record was made of this condition. However, we do not believe, with Weigert (1881) or

with Halpert and Coman ('30), that such a variation can be a major factor in the production of left superior vena cava. One who has examined the course of the dural sinuses in a number of skulls realizes that the passage of the superior sagittal sinus to the left at the confluence of sinuses is not an unusual occurrence. On the other hand a left superior vena cava, especially in the absence of the right, is a rare anomaly.

A microscopic study to determine a possible disturbance of location of the sinu-atrial node has not been made in our case. Dietrich ('13) and Mönckeberg ('21) have considered this matter. Mönckeberg criticizes certain of Dietrich's conclusions because of other pathology shown by the latter's case.

Because of the great enlargement of the mouth of the coronary sinus, it is evident that some displacement of the atrio-ventricular node also is inevitable.

We wish to express our thanks to Dr. Samuel Sanes for his assistance in the pathological interpretations, and to Dr. Niels C. Klendshoj for his translation of the paper by Schmidt.

LITERATURE CITED

- BEATTIE, J. 1931 The importance of anomalies of the superior vena cava in man. *Canad. Med. Assoc. J.*, vol. 25, pp. 281-284.
- BEDART 1892 Quelques cas rares d'anomalies musculaires observés à Toulouse au Laboratoire d'anatomie. 5°. Veine cave supérieure située à gauche. *Bull. Soc. d'anthrop. Paris*, T. 3, 4^e Serie, p. 379.
- BENDA, C. 1924 *Venen*. Henke und Lubarsch's Handbuch d. speziellen pathologischen Anatomie und Histologie. Julius Springer, Berlin, pp. 793-794.
- BOYD, S. 1893 Case of left superior cava without transposition of viscera. *Proc. Anat. Soc. Great Britain and Ireland. J. Anat. and Physiol.*, vol. 27, pp. XX-XXI.
- CHARLES, J. J. 1889 Notes of a case of persistent left superior vena cava, the right superior vena cava being in great part a fibrous cord. *J. Anat. and Physiol.*, vol. 23, pp. 649-650.
- CHESELDEN, W. 1713 Some anatomical observations. *Philosophical Transactions*, vol. 28, pp. 281-282.
- DIETRICH, A. 1913 Über ein Fibroxanthosarkom mit eigenartiger Ausbreitung und über eine Vena cava sup. sinistra bei dem gleichen Fall. *Virchows Arch.*, Bd. 212, S. 119-139.
- GORZULOFF, G. I. 1910 Left superior vena cava, the right being absent. *Universitetskiya Izvestiya, Kiyev*, vol. 50, no. 7, pp. 1-29. (In Russian.)

- GREENFIELD, W. S. 1876 Persistence of left vena cava superior, with absence of right. *Trans. Path. Soc. London*, vol. 27, pp. 120-124.
- GRUBER, W. 1880 Anatomische Notizen. VI (CLVI) Vorkommen einer Vena cava superior sinistra (bei Abwesenheit der V. cava superior der Norm). *Virchows Arch.*, Bd. 81, S. 458-462.
- HALBERTSMA, H. J. 1862 Abnormität der Vena cava superior. *Nederl. tijdschr. v. geneesk.* VI, p. 610. Reviewed in *Schmidt's Jahrb. der in- u ausländ. gesam. Med.*, 1863, Bd. 118, S. 163-164.
- HALPERT, B. 1927 Complete situs inversus of vena cava superior. *Anat. Rec.*, vol. 35, p. 38 (abstract).
- HALPERT, B., AND F. D. COMAN 1930 Complete situs inversus of the vena cava superior. *Am. J. Path.*, vol. 6, pp. 191-198.
- HUFFMIRE, A. P., AND G. C. BOWER 1919 A case of persistence of the left superior vena cava in an aged adult. *Anat. Rec.*, vol. 17, pp. 127-129.
- KEYES, D. C., AND H. C. KEYES 1925 A case of persistent left superior vena cava with reversed azygos system. *Anat. Rec.*, vol. 31, pp. 23-26.
- MÄUSER, A. 1899 Zur Kasuistik der Vena cava superior sinistra und der einen Spitzenlappen der Rechten Lunge abschnürenden Anomalie der Vena azygos. *Inaug. Diss.*, Giessen., S. 1-28.
- MCCOTTER, R. E. 1916 Three cases of the persistence of the left superior vena cava. *Anat. Rec.*, vol. 10, pp. 371-383.
- MÖNCKEBERG, J. G. 1921 Das Verhalten der Sinusknotens bei Fehlen der Vena cava superior dextra und Persistenz der Vena cava superior sinistra. *Beitr. path. Anat.*, Bd. 69, S. 537-548.
- NÜTZEL, H. 1914 Beitrag zur Kenntniss der Missbildungen im Bereiche der oberen Hohlvene. *Franf. Ztschr. Path.*, Bd. 15, S. 1-19.
- SCHMIDT, F. T. 1871 Aben vena cava sup. sin. med Obliteration af vena cava sup. dext. *Nord. Med. Ark.*, Stokholm, vol. 3, pp. 1-8.
- SCHÜTZ, H. 1914 Einige Fälle von Entwicklungsanomalie der Vena cava superior, (Persistenz des Linken Ductus Cuvieri.) *Virchows Arch.*, Bd. 216, S. 35-45.
- SMITH, W. C. 1916 A case of a left superior vena cava without a corresponding vessel on the right side. *Anat. Rec.*, vol. 11, pp. 191-197.
- DE LA VILLA, D. J. 1926 Un caso de vena cava superior izquierda. *Siglo Med.*, vol. 78, pp. 429-433.
- WEIGERT, CARL 1881 Kleinere Mittheilungen. 2. Ueber einen Fall von links verlaufender Vena cava superior muthmaasslich bedingt durch frühzeitige Synostose der Sutura mastoidea dextra. *Virchows Arch.*, Bd. 84, S. 184-188.

THE EFFECT OF MALE HORMONE UPON THE DESCENT OF THE TESTES ¹

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ONE PLATE (FIVE FIGURES)

Male hormone substances are known to affect the growth, maintenance and function of the male accessory organs of reproduction and secondary sexual characteristics. These hormones are also capable of maintaining spermatogenesis (Walsh, Cuyler and McCullagh, '34; Nelson and Gallagher, '36; Hamilton, '36) and of influencing cryptorchid testicles (Hamilton and Leonard, '37) in the hypophysectomized rat. The present paper presents evidence that male hormones are also implicated in the phenomenon of testicular descent.

Although sterility has been long recognized as a sequel of cryptorchidism, the significance and necessity of the scrotum as a thermoregulator for spermatogenesis was not appreciated until after the illuminating experiments by Moore and co-workers ('32). Shapiro ('30) in humans and Engle ('32) in monkeys demonstrated that anterior pituitary-like hormones were able to induce testicular descent, a lead that has been followed by many clinical investigators (Goldman and Stern, '33; Aberle and Jenkins, '34; Werner, Kelling, Ellersieck and Johns, '36). The data of the present paper show that male hormone substance is an agent actually responsible for growth processes of the reproductive organs which result in descent of the testes, and indicate that descent of retained testes obtained by anterior pituitary-like substances may be due in part at least to the production of male hormone substance.

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MATERIAL AND METHODS

As described by Wislocki ('35) and utilized by Engle ('32) and others, the immature monkey, *Macacus rhesus*, exhibits a cryptorchid condition from soon after birth until the onset of maturity, which is at the age of 4 to 5 years. Thus, the macaque between the age of 1 to 3 years and under 3 kg. body weight affords a naturally-occurring state of cryptorchidism upon which to test the efficacy of substances purported to cause testicular descent.

Twelve monkeys were given injections of testosterone acetate or testosterone propionate² in dosages of 1, 2, 3, 5 or 15 mg. Trial was made of the effect of varying the frequency of injections from two to seven times weekly. One cubic centimeter of peanut oil was used as a standard vehicle, since the type and amount of oil influences the absorption of the hormone (Parkes, '36).

Six monkeys were kept as controls, receiving the same dose of the oil vehicle at the intervals of administration employed in the experimental animals. The testes of one of these monkeys were almost completely descended, although the animal weighed only 2.8 kg. and had not received injections. The testes of the other five animals were as those of the experimental animals previous to injection (figs. 1 and 2) in that they rested about 4 to 6 cm. above the scrotum. Upon palpation they could be moved with ease 1 or 2 cm. caudally but returned spontaneously to a high cryptorchid site after palpation was completed. The scrota were flat, small and unpouched (figs. 1 and 2).

OBSERVATIONS

1. *Descent of testes.* In all but one of the eight monkeys which received doses of 5 mg. or more descent of the testes occurred within 3 weeks of treatment, usually about the fifteenth day after the beginning of treatment in those animals which received daily injections (figs. 3, 4 and 5). Four

² Testosterone acetate and testosterone propionate under the trade name Perandren were furnished through the courtesy of the Ciba Company.

animals which received 1 to 3 mg. doses twice weekly showed partial or no descent in 60 days. These doses which were insufficient to cause descent were also ineffective in producing the nasal changes of swelling and congestion in the area of the upper turbinate. Increase of the dose to 15 mg. administered at the same interval (twice weekly) produced descent in all four animals within 3 weeks and was accompanied by pronounced nasal changes (Mortimer, Wright and Collip, '36 a and b; Hamilton, '37 c).

Injections of 5 mg. and 15 mg. respectively were continued in three monkeys for 100 days. The testes remained in descent and the animals showed continued response to the androgenic material in the form of growth, maintenance and function of the accessory reproductive organs. This included a tendency toward a penile erection (Hamilton, '37 b).

When an animal was frightened the testes were on occasion retracted from the scrotum, but were returned when the animal relaxed or sat upon his haunches. In two monkeys which were not sacrificed at the end of the period of injections, the testes reapproached but never quite reached the original cryptorchid condition. A second series of injections with androgenic substance resulted in a more rapid lowering of the testes than in animals which had not previously received injections.

2. *Growth of accessory structures.* Preceding the descent there was enlargement of the prostate, seminal vesicles, penis, cord elements, and scrotum. The length of the inguinal structures enclosed by the cremaster muscles was 2 to 5 cm. in the uninjected monkeys, 5 to 7 cm. in injected animals. Gross enlargement and hyperplasia of the cremaster muscle, vas deferens, epididymis and attendant blood vessels were similar to, but of somewhat slighter degree than, the growth of these structures depicted after administration of anterior pituitary extracts (Deming, '36). The scrotum was usually transformed from a flat transverse band into a bilobed sac more pouched, dependent and rugose. The raphe became red and prominent. Sometimes the scrotum contained a small amount

of mucoid-like matter, but the edema and mucoid-like material never resulted in an approach to the great degree of scrotal swelling observed after administration of pituitary extracts (Engle, '32). Nevertheless, the more caudal position of the testes, their retainment in the scrotum, and the elongation of the inguinal structures, all of which followed androgenic injections, are interpreted by the author as evidence of testicular descent. Further indication of an effect of androgens on descent was in the lowering of testes when injections were given, elevation of the testes when injections were stopped and descent again when administration was begun once more.

DISCUSSION

Although an understanding of the embryological processes involved in the normally-occurring descent of the testes is necessary for an appreciation of the influence of male hormones upon testicular descent, such discussions must be left to other sources save to state that these processes can be hormonally influenced and even the processus vaginalis affected (Burrows, '36).

Testicular retention can be charged to mechanical, physiological and hormonal causes. Under mechanical may be listed such factors as adhesions, obstructions, strictures of the abdominal rings and faulty gubernacular attachments. Physiologically, reflex contraction of the cremasteric muscle is capable of intermittently returning the testis through a patent external ring into the inguinal canal, where secondary retention may take place and masquerade as organic cryptorchidism. Under hormonal causes may be considered hypodevelopment 1) of cord structures and accompanying blood vessels (plus in the monkey at least, the cremaster muscle) which are of insufficient length to permit lowering of the testes to a scrotal site, and 2) of the scrotal sac which may be so rudimentary as to afford little pocket to hold the testes. Surgeons are agreed that underdevelopment of inguinal structures and scrotum offers the two chief difficulties to successful orchiopexy; hence it is significant that this underdevelopment may be

treated hormonally either by pregnancy urine extracts (Shapiro, '30) or by male hormone substance. Presumably the pregnancy urine extracts act in part by stimulating the testes to produce male hormone substances or their precursors which can be agents at least in part actually responsible for testicular descent (Hamilton, '37 a). Advantages of synthetic male hormone substances over anterior pituitary-like material (pregnancy urine extracts) have been described briefly elsewhere (Hamilton, '37 b). Evaluation of androgenic substances in humans will be reported in the near future, but it should be remembered that critical study (Thompson et al., '37) of the descent obtained by gonadotropic substance revealed less than one-fourth of truly cryptorchid cases responded to endocrine treatment. It would seem optimistic to expect any high percentage of descent with androgenic substance; presumably mechanical causes account for a large number of cryptorchid cases and androgenic material should be expected to induce descent only where hormonal and perhaps physiological factors are at fault. Further, it is necessary that caution be used in the selection of human cryptorchidic cases, for spastic retraction of the testicle or false cryptorchidism forms a large percentage of the cases diagnosed as cryptorchid (Hamilton and Hubert, '37). Of the first sixteen patients referred by pediatricians to the author, ten were but cases of spastic retraction. Surgery, hormonal treatment or merely waiting until tomorrow might effectively produce descent. One wonders how much of the success of some methods has been in part due to the use of these cases, as when a testis has been said to descend within 3 hours after hormonal treatment (Harris, '35). It would seem well to use special means (Hamilton and Hubert, '37) to differentiate true from pseudo-cryptorchidism, although in the few cases of intermittent retention studied by the author, androgenic substance has been also found of value in that retraction is minimized or prevented following treatment.

Thus, the administration of hormone substances to the young cryptorchid child to produce an early and an accentuated 'pubertal state' may prove of value 1) in producing non-operative descent in some cases (especially such as those of hypopituitarism where endocrine stimulation has been lacking) and in avoiding waiting until puberty to ascertain whether or not spontaneous descent may occur 2) in selecting for early operation those cases where descent is prevented because of mechanical or other factors; thus, hernia, malignancy, testicular atrophy, pain, anxiety, and other conditions possibly attendant upon cryptorchidism may be minimized; and 3) in aiding surgery pre- and post-operatively in the development of the cord elements and scrotum.

SUMMARY

1. Cryptorchid testes of the immature macaque have been observed to descend into the scrotum following adequate administration of the male hormones, testosterone acetate and testosterone propionate. There is less edema and scrotal swelling than in descent produced by gonadotropic substance.

2. Processes considered largely responsible in the production of this descent are 1) growth and elongation of cord elements and 2) slight development of the scrotum to form more of a pouch for retainment of the testes.

3. It is suggested that descent produced by anterior pituitary-like substances may be in part due to stimulation of the secretion of male hormone substances.

4. Hormone treatment may be of value in a) producing descent in certain cryptorchid cases, b) authorizing surgery at an early age instead of waiting until puberty to see if spontaneous descent might occur; thus, some of the objectionable phenomena attendant upon cryptorchidism may possibly be avoided, and c) aiding surgery pre-operatively by the development of cord structures and post-operatively by preventing retraction and tension.

LITERATURE CITED

- ABERLE, S. B., AND R. H. JENKINS 1934 Undescended testes in man and rhesus monkeys. *J. Am. Med. Assoc.*, vol. 103, pp. 314-318.
- BURROWS, H. 1936 Influence of oestrogenic compounds in causing hernia and descent of testis in mice. *Brit. J. Surg.*, vol. 23, pp. 658-664.
- DEMING, C. L. 1936 The gonadotropic factor as an aid to surgery in the treatment of the undescended testicle. *J. Urol.*, vol. 36, pp. 274-288.
- ENGLE, E. T. 1932 Experimentally induced descent of testis of *Macacus* monkey by hormones from the anterior pituitary and pregnancy urine; the role of gonadokinetic hormones in pregnancy blood in the normal descent of the testis in man. *Endocrinology*, vol. 16, pp. 513-520.
- GOLDMAN, A., AND A. STERN 1933 Treatment of undescended testes by injection of prolan. *New York State J. Med.*, vol. 33, pp. 1095-1096.
- HAMILTON, J. B. 1936 Endocrine control of the scrotum and a 'sexual skin' in the male rat. *Exp. Biol. and Med.*, vol. 35, pp. 386-387.
- 1937 a Production of testicular descent in monkeys by male hormone substance. *Am. J. Physiol.*, vol. 119, p. 325.
- 1937 b Induction of penile erection by male hormone substances. *Endocrinology*, vol. 21, pp. 744-749.
- 1937 c Changes in nasal mucosa of monkeys (*Macaca rhesi*) and humans by male hormone substances. *Proc. Soc. Exp. Biol. and Med.*, vol. 37, pp. 366-369.
- HAMILTON, J. B., AND G. HUBERT 1937 Differential diagnosis of pseudoorchidism and true cryptorchidism. *Endocrinology*, vol. 21, pp. 644-648.
- HAMILTON, J. B., AND S. L. LEONARD 1938 The effect of male hormone substance upon the testes and upon spermatogenesis. (In press.)
- HARRIS, F. I. 1935 Treatment of undescended testicle. *Am. J. Surg.*, vol. 27, pp. 447-454.
- MOORE, C. R. 1932 Sex and Internal Secretions. Edited by E. Allen. The Williams and Wilkins Company, Baltimore.
- MORTIMER, H., R. P. WRIGHT AND J. B. COLLIP 1936 a The effect of the administration of estrogenic hormones on the nasal mucosa of the monkey (*Macaca mulatta*). *Canad. Med. Assoc. J.*, vol. 35, pp. 503-513.
- 1936 b The effect of oestrogenic hormones on the nasal mucosa; their role in the naso-sexual relationship, and their significance in clinical rhinology. *Canad. Med. Assoc. J.*, vol. 35, pp. 615-621.
- NELSON, W. O., AND T. F. GALLAGHER 1936 Some effects of androgenic substances in the rat. *Science*, vol. 84, pp. 230-232.
- PARKES, A. S. 1936 Increasing the effectiveness of testosterone. *Lancet*, vol. 2, pp. 674-676.
- SHAPIRO, B. 1930 Kann man mit Hypophysenvorderlappen den unterentwickelten männlichen Genitalapparat beim Menschen zum Wachstum anregen? *Deutsche med. Wchnschr.*, vol. 56, pp. 1605-1607.
- THOMPSON, W. O., A. D. BEVAN, H. J. HECKEL, E. R. MCCARTHY AND P. K. THOMPSON 1937 Treatment of undescended testes with anterior pituitary-like substances. *Endocrinology*, vol. 21, pp. 220-229.

- WALSH, E. L., W. K. CUYLER AND D. R. McCULLAGH 1934 The physiologic maintenance of the male sex glands. *Am. J. Physiol.*, vol. 107, pp. 508-512.
- WERNER, A. A., D. KELLING, D. ELLERSIECK AND G. A. JOHNS 1936 Effect of gonadotropic extract of the pituitary in cryptorchidism. *J. Am. Med. Assoc.*, vol. 106, pp. 1541-1543.
- WISLOCKI, G. B. 1935 Observations on the descent of the testes in the macaque and in the chimpanzee. *Anat. Rec.*, vol. 57, pp. 133-148.

PLATE 1

EXPLANATION OF FIGURES

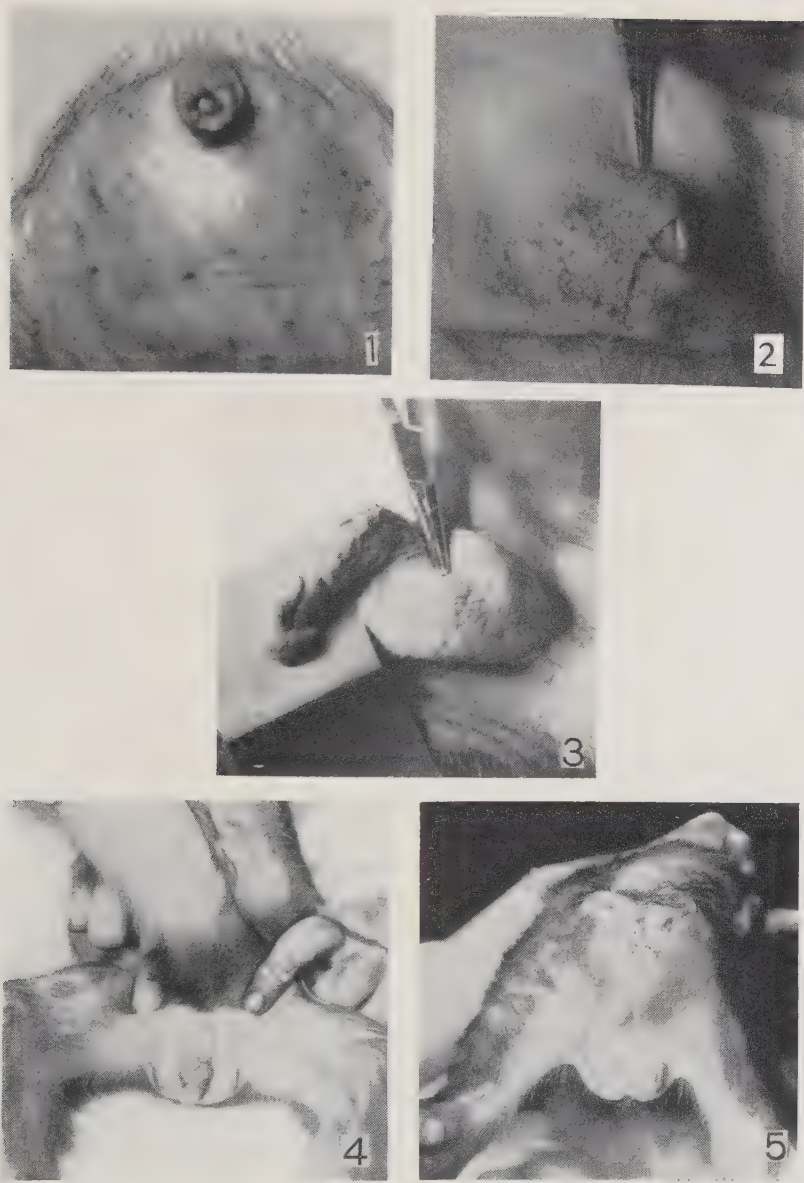
1 Flat, empty scrotum and cryptorchid condition of an immature untreated macaque of 2.0 kg.

2 Ventral view of cryptorchid condition in an immature untreated macaque of 2.2 kg. Note the testes lie in a high inguinal position on either side of the pointer.

3 Testicular position of testes following treatment of an 1.8 kg. macaque with daily injections of 15 mg. of testosterone propionate in peanut oil for 25 days. The penis and scrotum show development and adult characteristics. Note the glans penis and scrotal rugae. The raphe has become red.

4 Descended testes of 1.7 kg. animal which received 5 mg. of testosterone propionate for 40 days. Note the enlarged scrotum. The bulging lying lateral to each side of the penis is the cremaster muscle ensheathing the elements of the cord. Note development of the penis and frenulum, the latter and the scrotal raphe being red-pink in color.

5 Rear view; 1.9 kg. androgen-injected monkey, illustrating pendulous scrotum and the testes contained with the scrotum. The androgen dosage was 5 mg. of testosterone acetate daily for 40 days.



THE GOLGI APPARATUS OF THE CELLS OF THE ADRENAL CORTEX AFTER HYPOPHYSECTOMY AND ON THE ADMINISTRATION OF THE ADRENOCORTICOTROPIC HORMONE

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TEN FIGURES

The gravimetric increase in the adrenal of normal rats following administration of the adrenocorticotrophic hormone of the anterior hypophysis is well known, and has been proposed as a basis for a method of assay (Moon, '37). We felt that it might be of interest to determine whether or not the Golgi apparatus in the adrenal cortical cells manifested any change in morphology as a result of administration of the adrenocorticotrophic hormone. We postulated that some change in the apparatus might be effected by hypophysectomy.

Bourne ('34) has briefly reviewed the earlier literature quoted by Rogoff ('32) (Pensa, 1899; Ricci, '24; Vincent and Curtis, '27; Merland, '29). Bourne examined the Golgi apparatus of the adrenal in the opossum, the Australian flying phalanger and the young rat. Since our own work has been confined to the male rat it is Bourne's remarks concerning that animal which are of most interest here.

Bourne found that in "the adrenals of young rats from twenty-four hours to eight days old, the cells of the zona glomerulosa showed the Golgi apparatus in the form of a small black knot with branches extending over the nucleus." In some cells of the zona fasciculata he found the Golgi apparatus in the form of granules. "In some cells there was a very dense aggregation of lipoid globules within the cell and

scattered between them were a number of granules which were presumably Golgi material; no formed apparatus could be seen."

Bourne states that "this association of the black granules and lipoid globules made these cells appear dark in contrast with other cells of the cortex in which there are fewer lipoid globules and a true apparatus associated with the nucleus." In the adrenals of a young rat 2 weeks old "the three zones of the medulla (cortex?) showed the presence of typical forms of the Golgi situated like caps over the nuclei, always on the side near the distal portion of the cell." In the medulla Bourne found the Golgi apparatus "invariably in the form of a cap over one side of the nucleus sending branches over it, not oriented in any particular direction." Nahm and McKenzie ('37) have noted the intimacy of the Golgi apparatus and the nucleus in the adrenal cortex of the ewe, as has Ivanov ('32) in a number of other forms.

MATERIAL AND METHODS

In the demonstration of the Golgi apparatus we have relied entirely on the method described by Da Fano. A silver technique was used because, as Bourne has pointed out, this lessens the possibility of "the Golgi material being confused with the free lipoid of the cortical cell."

Hoerr ('31) has pointed out the difficulty of obtaining adequate fixation in the adrenal cortex. Admittedly cobalt-formol fixation is inadequate in many respects; however, since this study is restricted to the Golgi apparatus such procedure is justified.

We have fixed material in the cobalt nitrate-formol mixture made up both in distilled water and in physiological salt solution. While, as Bourne contends, there may be somewhat better preservation of cell contour with the saline fixative, we can distinguish no advantage as far as fixation of the Golgi apparatus is concerned. Some of our most uniform preparations have been obtained with the ordinary Da Fano fixative using 10% formol. The time devoted to fixation has

been 3 to 4 hours. Silvering and reduction has been carried out in the dark.

To rule out the possibility of confusion of the Golgi apparatus with vitamin C granules we have followed Bourne in studying some of our material before reduction. We feel that the possibility of such confusion is negligible.

We have investigated the adrenals of the following series of male rats:

1. A series of normal immature male rats, 24 to 27 days of age, this being the approximate age of the animal used for testing the adrenocorticotrophic potency of the solutions used.

2. A series of normal mature males 59 days of age.

3. A series of hypophysectomized males operated when mature and autopsied at periods varying from 28 hours to 377 days following operation.

4. A series of normal males 24 to 27 days old which received a single dose of two ¹ units of adrenocorticotrophic hormone, which animals were autopsied at approximately 24 hours following administration of the hormone.

5. Animals which received a total dose of the adrenocorticotrophic fraction given in three equally divided doses over a period of 3 days. The animals were sacrificed 24 hours after the last injection.

6. As a final study we have given the adrenocorticotrophic hormone as substitution therapy to hypophysectomized animals. Administration was begun 24 hours after operation and 0.75 unit was given per dose. Eight days after operation and 24 hours following the final injection the animals were autopsied. The total dose administered was 5.25 units.

OBSERVATIONS

Our observations on the adrenal of the normal male rat parallel those of Bourne for the most part. In the adrenal

¹ "The amount of adrenocorticotrophic hormone necessary to cause an increase of 50% over the adrenal weight of controls when injected into 21-day-old male rats in 3 doses over a period of 3 days was defined as one normal rat unit." (Moon, '37.)

cortex of the rat we found a 'type' Golgi apparatus which manifests numerous variations. This apparatus is one in which the Golgi material occupies a juxta-nuclear position and has the form of a pyramid or cap. The pyramid sometimes appears quite solid and homogeneous but again there may be seen in it numerous spaces. Whether we are to regard these spaces as vesicular inclusions in the central knot or whether they represent inter-granular intervals, the result is to impart to the apparatus in most instances the appearance of a close aggregation of granules.

Extensions from the central knot are superimposed upon the nuclear membrane, and extend over that membrane as finger-like projections. These extensions vary somewhat in density, but in the normal animal, they are usually quite fine. Frequently they are composed of rows of argentophile granules which decrease in size as the projection is traced distally from the central knot. In the normal gland we seldom find anastomoses between these projections with the formation of a true network. In some cells the projections seem quite homogeneous in composition. The Golgi apparatus when seen in polar view usually presents a stellate appearance. In addition, small spherules of Golgi material may be seen lying in apposition to the nuclear membrane at intervals about its circumference. These spherules are sometimes connected by extremely delicate strands external to the nuclear membrane. This 'type' Golgi apparatus has many modifications in the cortex of the normal animal. It varies from zone to zone and in the cell types to be described.

The cytoplasm of the adrenal cortical cells varies in the capacity with which it takes up the silver. This gives rise to two classes of cells on the basis of cytoplasmic reaction. One of these classes of cells has a light cytoplasm while the other, which appears to contain more lipid vacuoles is by contrast dark in appearance. We presume that the lightly and darkly silvered cells thus demonstrated are probably not the counterpart of the light and dark cells described when other methods of fixation and staining are used. Their distribution is not consistent with those described in the guinea pig

by Hoerr. The darkly silvered cells we have found in abundance in the outer fasciculata and a similar type of cytoplasmic reaction is sometimes found in the inner fasciculata and reticularis. These findings in the inner fasciculata and reticularis are not constant.

The glomerulosa cells of both the immature and the mature male rat present a Golgi apparatus which appears as a black knot lying in apposition to the nucleus. The apparatus in this situation is frequently over impregnated, but in optimum preparations it can be seen to have perinuclear extensions such as those above described. We are not convinced that the apparatus in the glomerulosa has any constant orientation relative to the periphery of the gland.

From the work of Dornfeld ('36) one would infer that the Golgi apparatus in the zona fasciculata in the rat adrenal was always in a dispersed state. We have found that such is by no means the case. Dornfeld states that "In the fasciculata cells of the cortex, indeed, there most usually appear in sections only osmiophilic granules between the lipoid droplets: a highly diffuse form of the apparatus, though looked upon as a precipitation artifact by Golgi critics."

There can be no doubt that in many of the fasciculata cells the Golgi apparatus reaches a considerable degree of dispersal; particularly, is this true in those cells whose lipoid content is high. Some cells have indeed no formed apparatus. We are convinced however that a Golgi mass associated with the nucleus is the rule, even though there be a considerable amount of Golgi material diffused throughout the cytoplasm.

In the immature male rat those cells of the fasciculata whose cytoplasm appears light by contrast, have a Golgi apparatus which conforms more completely to the 'type' apparatus than does that of the darkly silvered cells. In the darkly silvered cells the form of the Golgi apparatus is quite variable. The Golgi material in the majority of these darkly silvered cells manifests a greater degree of complexity and its total mass is somewhat greater than that found in the lighter cells. We have noted the fact that in the dark cells the projections from

the central juxta-nuclear knot extend into the cytoplasm to a marked degree. In these markedly vacuolated cells, which vacuoles presumably contained lipoid, we have often noted that the Golgi apparatus is drawn out in strands between the vacuoles from the nuclear cap. In the fasciculata we have seen strands of Golgi material apparently lying free in the cytoplasm of the darkly silvered cells. A phenomenon rarely observed in this cell type is a Golgi apparatus as a rounded compact mass lying near the nucleus but not a true nuclear cap. Such cells do not show any extensive vacuolization. In many of the cells of presumably high lipoid content the Golgi apparatus appeared to be undergoing the 'hypertrophy and fragmentation' described by Bourne.

In the innermost fasciculata and reticularis the character of the Golgi apparatus varies definitely from that seen in the outer zones. Bourne has described similar findings in *Petaurus breviceps* and *Trichosurus vulpecula* but has not described them in the rat.

In the juxta-medullary cortex (the innermost fasciculata and reticularis being ill-defined in the rat) the Golgi apparatus is definitely smaller in size than in the outer zones. The elements composing the apparatus in this area are more definitely granular than in the outer zones, and there is a trend toward greater variation in the size of the granules. One is impressed by the scarcity of definite perinuclear extensions. The Golgi apparatus often seems to be retracted into a rather circumscribed granular body, pyramidal or spherical in shape, lying near the nucleus.

These findings (similar to those of Bourne) would agree insofar as the Golgi apparatus is concerned with the conception that the inner zone is one of senescent cells.

Comparison of the adrenals of the mature and immature male shows certain minor differences. In the mature animal the Golgi material is somewhat more abundant in the fasciculata cells. Particularly is this true of the darkly silvered cells. These cells in particular exhibit that process described by Bourne as 'hypertrophy and fragmentation.' Extensions of

the apparatus lying in apposition to vacuoles are of frequent occurrence. The zona reticularis and inner fasciculata show granulation and retraction of the Golgi material to even a more marked degree than that seen in the immature male. Although there is some variation from animal to animal and from cell to cell in the same animal, the Golgi apparatus of the medulla is typically a nuclear cap in both mature and immature animals.

In animals hyophysectomized for 28 hours it is evident that the Golgi material in the adrenal cortical cells has undergone a definite change. In the fasciculata where the morphology of the apparatus was so varied in the normal mature male, many cells show a condensation, or shrinkage of the apparatus into a mass which lies near the nucleus. Many of the cells show, even at this early date, definite evidence of loss of the digital projections which normally lie upon the nuclear surface. Here, too, there begins to be apparent a difference in the type or argentophile substance composing the Golgi apparatus. The apparatus in the fasciculata cells is more definitely granular than that seen in the normal adrenal. When contrasted with the relatively large spherules sometimes seen aggregated as the apparatus of the normal cell these granules tend to be fine and more irregular. This does not apply to all cells at this stage following operation, but many cells are affected and the change in the general picture is definite. In the glomerulosa the apparatus is still seen in the form of juxta-nuclear knots. In the medulla, the apparatus appears in many cells as a typical nuclear cap, and in other cells it is less well organized, but there is no obvious deviation from the normal.

In animals sacrificed 3 days following operation the change in the picture is more marked and the contraction of the apparatus into a circumscribed granular structure is more definite. Some of the lipoid-filled, darkly silvered cells still show a moderate degree of complexity of the apparatus, but, for the most part, shrinkage, increased granularity and loss of nuclear extensions are present in both types of cells. The Golgi apparatus throughout the cortex approximates the form seen normally in the reticularis and inner fasciculata.

In these animals the glomerulosa showed the apparatus in the form of heavily impregnated black knots, while the cells of the juxtamedullary cortex showed an apparatus which was very granular and compact.

At 5 days post-operative, it was found that the process outlined above was even more marked. In many of the cells of the fasciculata the granular aggregate forming the apparatus was more closely packed than in the earlier post-operative stages. This massing together of the granules in some cases resulted in the formation of a small knot lying near the nucleus. Although in some cells the structure appeared radiate the projections were not sent out over the nucleus as typical nuclear extensions. In some cells the juxta-nuclear granular material seemed to be immersed in a hyaline substrate. The Golgi apparatus of the medullary cells showed no remarkable features.

In animals examined 7 days after hypophysectomy the picture was essentially similar.

All drawings made with aid of camera lucida and taken from the zona fasciculata of adrenal cortex of the male rat.

Fig. 1 Darkly silvered cell from immature rat. Golgi apparatus juxta-nuclear with extensions over nuclear surface.

Fig. 2 Lightly silvered cell from immature rat.

Fig. 3 Lightly silvered cell from mature rat. Golgi apparatus a nuclear cap with extensions upon nuclear surface.

Fig. 4 Darkly silvered cell from mature rat showing the radiation of Golgi material from the central knot out between lipoid vacuoles.

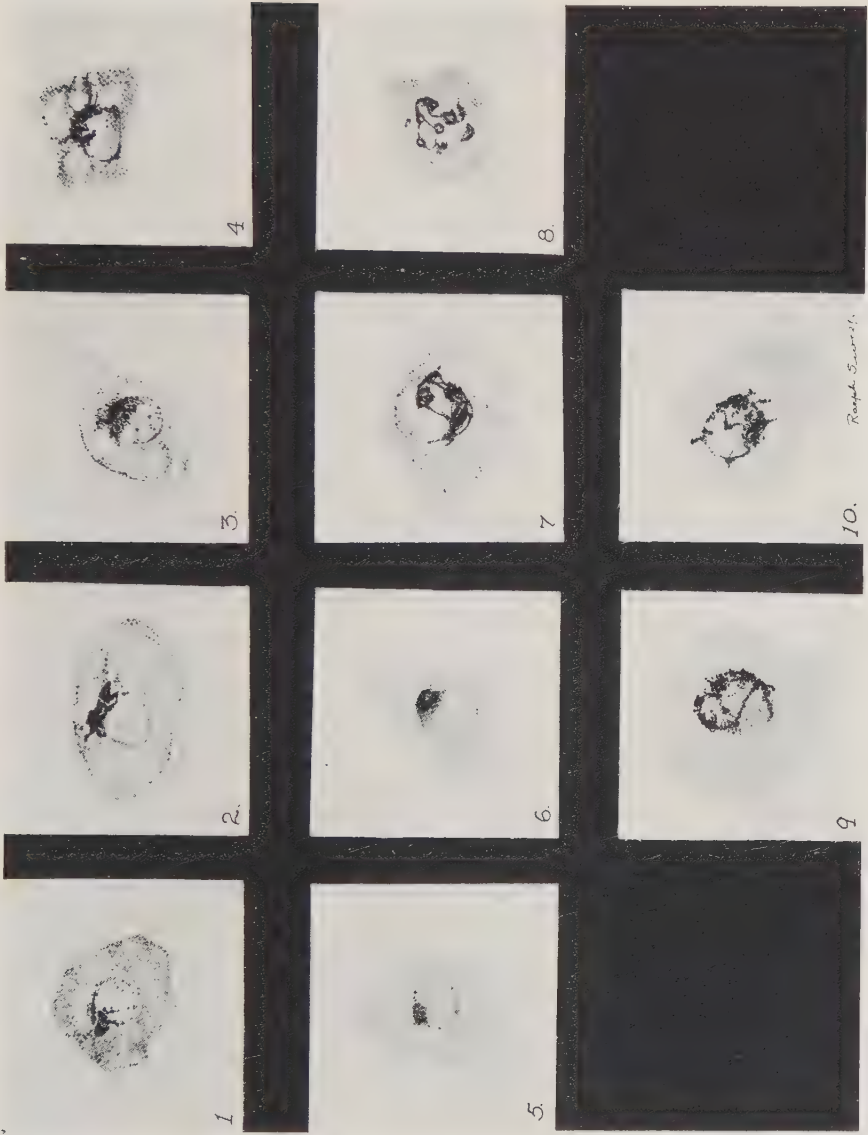
Fig. 5 Cell from a rat hypophysectomized 5 days. Golgi apparatus shows granulation and shrinkage.

Fig. 6 Cell from a rat hypophysectomized 377 days. Golgi apparatus still present as a shrunken juxta-nuclear mass. Fusion of granular material evident.

Fig. 7 Cell from normal rat after administration of 2 units of adrenocorticotrophic hormone. Note moderate hypertrophy of Golgi apparatus and extension of same about nucleus.

Fig. 8 A more marked stage of hypertrophy than in figure 7. The cell is chosen from the same material.

Figs. 9 and 10 Cells showing hypertrophy of Golgi apparatus in hypophysectomized animals following administration of the adrenocorticotrophic hormone. (There is more cytoplasmic granulation in this material than is evident in these two cells.)



In two animals examined 21 days following operation, granularity of the Golgi material was evident in the zona fasciculata, but there was marked evidence of increasing density of the apparatus. The granules in many instances were shrunken together into a compact knot. In one of these animals it was noted that in numerous cells there seemed to be fusion of the granular material into strands which led to the appearance of a minute net-like structure lying on one side of the nucleus. Again, the Golgi apparatus of the cells of the medulla showed no radical departure from that seen in the normal gland. Simultaneous with the shrinkage of the Golgi apparatus there appears a progressive homogeneity in the cytoplasm of the adrenal cortical cells. The silver imparts to the cytoplasm in normal adrenal cortical cells a definitely granular appearance, the granules varying remarkably in size. This appearance tends to be lost following hypophysectomy. One might infer that the loss of this granular material was correlated with diminution in secretory function.

We have examined the adrenals of three animals killed 377 days after hypophysectomy. In all of these the granularity of the Golgi apparatus is very apparent; the Golgi apparatus is reduced in size. Where this shrinkage is marked and if the impregnation is not too heavy, one may see fusion of granular material into strands with the formation of a minute net-like structure as is described above.

While in general the differentiation of the lightly and heavily silvered cells is less distinct in the hypophysectomized animals, in a rat hypophysectomized 377 days both cell types can be seen in the outer areas of the cortex.²

In a series of normal males, 24 to 27 days of age which had been given 2 units of the adrenocorticotrophic factor, the hypertrophy of the Golgi apparatus is remarkable. Because perhaps of the increased fat in these adrenals, uniformity of

² As this paper is completed we note the reference of Dornfeld ('37) to unpublished work in which he has found "retraction of the diffuse Golgi network into a dense juxta-nuclear mass" in the zona fasciculata of the rat adrenal following hypophysectomy. (*Anat. Rec.*, vol. 60, p. 229, '37.)

preparations was difficult to secure. A sufficient series, however, is at hand, to point out certain facts very definitely.

Within the short period of 24 hours the apparatus is transformed into a structure of great complexity. There is an increase in the total mass of the Golgi material present. While the central knot is in many cases, still present, the striking feature is the migration about the nucleus of heavy strands of argentophile material which often anastomose to form a true perinuclear net. While the degree of hypertrophy varies somewhat from cell to cell, and while accurate counts have not been made it is evident that most of the cells are thus affected. Many of these strands have a frothy, vacuolated appearance. Sometimes the strands consist of rows of argentophile granules joined by filaments.

The apparatus in the outermost zone of the fasciculata and the glomerulosa is adequately seen in few of these preparations. Where it is seen, however, the picture leaves no doubt that in these areas the apparatus undergoes the hypertrophy seen throughout the fasciculata except in the portion adjacent to the reticularis.

The innermost fasciculata and reticularis show, in general, much less evidence of hypertrophy of the Golgi material. Only in one gland did we find that there was definite evidence of Golgi hypertrophy in these zones. We feel that probably this is due to the inability of the senescent cells of this region to respond to the stimulus to the same degree as do the outer cells of the cortex.

In two normal animals of the same age, the total amount of 2 units of the adrenocorticotrophic hormone was given in three equally divided doses over a period of 3 days. The animals were autopsied at the end of the 3-day period. In the adrenal cortex of these animals, the Golgi apparatus showed definite evidence of stimulation as a result of the administration of the adrenocorticotrophic hormone. Particularly do we find stimulation in the outer third of the fasciculata. The reaction, however, was far less marked than in those animals which had received the total dose in one injection. Response was

evident in the apparatus in both the lightly and darkly silvered cells. One feature of the apparatus in these glands which deserves mention is that, particularly in the dark cells, the strands seem less well organized than in the 24-hour series. There is furthermore, a tendency toward extension of the Golgi material out into the surrounding cytoplasm which was not seen in those animals which received the total dose in one injection.

Two animals which had been hypophysectomized and in which administration of the hormone was begun 24 hours after operation, were autopsied on the eighth day post-operative. They were sacrificed 24 hours following the last administration of the adrenocorticotrophic material. The total dose given was 5.25 units.

It was clear, on examining the Golgi apparatus in these animals that the usual post-operative granulation and shrinkage was absent and that not only was their maintenance of the apparatus, but in the outer areas of the cortex there was definite evidence of hypertrophy and migration of the apparatus about the nucleus.

In one of these animals there was clear evidence that, consistent with our findings in the unoperated, injected animals, the innermost areas of the cortex evidenced no marked response.

DISCUSSION

It is not our intention to reopen the question of the relation of the Golgi apparatus to the formation of lipoid. The fact that the hypertrophy of the Golgi apparatus and an increase in lipoid in the adrenal cortex occur simultaneously is of some interest. The intimate association of the Golgi apparatus and the lipoid vacuoles seen in the normal animal, and previously noted by Bourne, constitute a fascinating subject for speculation, but since any conclusions would be based on inference we do not presume to draw any here. We hold the same attitude regarding the distinct change in the character of the cytoplasm following hypophysectomy.

The regressive change incident to hypophysectomy we feel signifies a diminution in some function of the adrenal cortical cell. The fact that the apparatus does not disappear but remains in its modified state for longer than a year after operation would agree with the conception that the cell carries on adequate function to maintain life, but that its activities are at a low ebb.

We do not feel justified in drawing any conclusions regarding the Golgi apparatus as an indicator of cell polarity in the absence of serial reconstructions, but on the basis of our studies no such indications are present.

The reaction to hypophysectomy and the reaction to specific stimulation of the cortex leaves no doubt that the Golgi apparatus in the adrenal cortex plays a very direct role in cell physiology and that this role is proportional to cell activity.

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SUMMARY

1. The morphology of the Golgi apparatus in the cells of the adrenal cortex of the male rat is described.

2. The Golgi apparatus particularly in the zona fasciculata undergoes a change in granularity and becomes shrunken following hypophysectomy, and it resembles the type of Golgi apparatus seen normally in the juxta-medullary cortex where the cortical cells are senescent.

3. Following the administration of the adrenocorticotrophic hormone to the normal animal of 24 to 27 days of age there is a marked hypertrophy of the Golgi apparatus and an extension of the apparatus about the nucleus. In the inner areas of the cortex where the cells in the normal adrenal are presumably senescent, the reaction to the stimulation is less marked.

4. Substitution therapy with the adrenocorticotropic hormone was begun in the hypophysectomized animal 24 hours after operation. As a result of such treatment, not only was the normal appearance of the Golgi apparatus in the adrenal cortex maintained, but the apparatus showed definite evidence of hypertrophy.

5. The Golgi apparatus of the medulla fails to show any consistent change which may be correlated with those occurring in the cortex.

LITERATURE CITED

- BOURNE, G. 1934 A study on the Golgi apparatus of the adrenal gland. *Australian J. Exp. Biol.*, vol. 12, pp. 123-139.
- DORNFELD, E. J. 1936 Nuclear and cytoplasmic phenomena in the centrifuged adrenal gland of the albino rat. *Anat. Rec.*, vol. 65, pp. 403-414.
- HOERR, N. 1931 The cells of the suprarenal cortex in the guinea pig. Their reaction to injury and their replacement. *Am. J. Anat.*, vol. 48, pp. 139-197.
- IVANOV, M. F. 1932 Tsitologicheskie nabludeniia nad koroii nadpochechnykh zhelez u mlekoptaiushchikh. (Cytology of the suprarenal cortex in mammals.) *Russkii Arkhiv Anatomii, Gistologii i Embriologii*, vol. 11, pp. 79-94. (German summary, pp. 219-222.)
- MOON, H. D. 1937 Preparation and biological assay of adrenocorticotropic hormone. *Proc. Soc. Exp. Biol. and Med.*, vol. 35, pp. 649-652.
- NAHM, L. J., AND F. F. MCKENZIE 1937 The cells of the adrenal cortex of the ewe during the estral cycle and pregnancy. *Univ. Miss. College Agricul. Research Bull.*, no. 251.
- ROGOFF, J. M. 1932 The suprarenal bodies. M. Cowdry. *Special cytology*, 2nd ed., pp. 869-931. Hoeber.

A REVIEW OF THE GOLGI APPARATUS

PART II

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3. IDENTIFICATION OF THE GOLGI APPARATUS

Probably the most serious source of error in Golgi apparatus work has been in identifying the apparatus. In the words of Hertwig (p. 261),

. . . . verfügen wir bisher nicht über ein eindeutiges Identifizierungsmerkmal für den Golgiapparat. Die Diagnose, ob ein unbekanntes Strukturgebilde als Golgiapparat bezeichnet werden darf, gründet sich vielmehr auf ein Anzahl verschiedener Merkmale, die einzeln nicht absolut charakteristisch und eindeutig sind. Es ist deshalb dem Forscher ein gewisser, an sich natürlich nicht erwünschter Spielraum gelassen, wie hoch

¹ Part I of this review appeared in the March issue of *The Anatomical Record*; part III will appear in the May issue.

er die einzelnen auf verschiedenem Wege erhaltenen Befunde gegeneinander abschätzen und zu einem Gesamtbilde verwerten will. Daraus erklären sich die vielen Widersprüche, die sich in der Literatur über das, was wir Golgiapparat nennen dürfen, finden, und die namentlich bei Pflanzlichen Zellen von einer Lösung noch weit entfernt sind.

The entire Golgi-trophospongium and Golgi-vacuome controversies are based upon differences of opinion as to what constitutes the Golgi apparatus in microscopic preparations. Much of this error must be attributed, of course to the fundamental difference of opinion concerning the relationships between vacuome and Golgi apparatus. However, many mistakes have arisen, not from erroneous theorizing, but from lack of specific tests for different cytoplasmic components, also from regarding such non-specific tests as osmic acid, neutral red and Janus green as specific. Even as nearly specific a stain as Sudan III has been employed in neutral red solution as a means for demonstrating intravitaly the lipoidal Golgi apparatus (Ma and Chang, '28), while what it actually demonstrates is in all likelihood the vacuome. The literature records instance after instance in which intracellular vacuoles or canals, preformed or induced (Covell and Scott, '28; Ma, '30),² prozymogen granules (Owens and Bensley, '29), intracellular lipoids (Nahm, '33), deposition of osmium on Nissl bodies (Legendre, '10), and mitochondria (Parat, '28) have been mistaken for Golgi material. Many more references might be mentioned here, but the bibliographies are included in the papers noted. Owens and Bensley ('29) declared that: "the osmic and silver impregnations are not sufficient by themselves to identify the Golgi apparatus or vacuome, since the available osmium or silver may be captured by other structures, leaving the Golgi apparatus unstained." Nahm ('33) expressed quite a different point of view: "Any substance either present in the living cell or capable of being produced from compounds in the living cell, which can reduce osmic acid may be Golgi substance." She is one of the few who maintain

² See reviews of Chlopin ('27), Guillermond ('27, '35), Parat ('28), Gatenby ('31 a).

that neither Golgi apparatus nor vacuome are structural entities in the living cell, but believes that "the vacuoles (neutral red vacuome) and Golgi elements are substances that appear during the transformations of protoplasmic constituents; they are the visible products of chemical reactions that occur in cells."³ Even as uncompromising a believer as the late Robert Bowen wrote ('26 a): "There is, therefore, no specific stain for the Golgi substance, and it cannot be rigorously defined by its behavior toward any known technical processes." Bowen continued with a statement which represents the current attitude: "This (difficulty of identification) must not be taken too pessimistically by those unversed in the niceties of cytological technique, for much the same facts can be stated for any cellular constituent. Indeed, the idea of specific stains whereby we may guide our footsteps through the intricate mazes of the cell has now pretty well gone to pieces." This expression is echoed by Gatenby ('31 a) who wrote: "Certainly, Wilson, Hirschler, Ludford, Bowen, the writer, and modern workers in general have experienced no difficulty in identifying Golgi bodies"; and again by Beams and King ('32): "We are in agreement with Gatenby in that we too have never been disturbed in making a decision as to the identity of Golgi material and mitochondria."

In spite of the imperfection of microchemical techniques, and notwithstanding an occasional dissenting voice, it seems likely that mistaking the identity of the Golgi apparatus in most vertebrate cells at least, is largely a thing of the past. (However, see Dawson, '31, who believed that "The problem of the identity of the Golgi substance appears to increase rather than decrease in complexity.")

4. POLARITY OF THE GOLGI APPARATUS

The position of the Golgi apparatus in the cell has been considered significant by many investigators. Among the first

³ See also Guthrie ('26), Just and McNorton ('27), Walker and Allen ('27), Tretjakoff ('28), Walker ('28), DeRoo and Ufford ('30), Tennent, Gardiner and Smith ('31), Payne ('32), Winters ('33), Sawyer ('36).

to study the problem was Golgi ('09), who followed changes in the position of the apparatus in the mucus-secreting cells of the stomach. Following Cajal's ('15) announcement that the Golgi apparatus in the ectodermal cells of the embryo always lies between the nucleus and the free surface of the cell, irrespective of alterations in the original relative positions of the germ layers, there has been a great abundance of observations upon the polarity of the apparatus.

In exocrine gland cells the apparatus has been observed by all investigators, between the nucleus and excretory pole. In the acinar cells of the pancreas in particular this polarity has been described so many times (Bensley, '11; Saguchi, '20; Nassonov, '23 a, '24; Bowen, '24 a, '26; Morelle, '25, '27; Beams, '30; Gatenby, '31 a; Hirsch, '31, '32; and numerous others) that there can be no doubt concerning its constancy.

In endocrine gland cells, on the other hand, the observations have been more uncertain and contradictory. Courier and Reiss ('22) and Reiss ('22), for the parathyroid and pituitary glands respectively, assumed that the Golgi apparatus is located at the excretory pole of the cell. Moreover, recently Rosof ('34) claimed that in the rat parathyroid the Golgi apparatus indicates secretory polarity and varies in its condition with the secretory activity of the cell. He considered the secretory stage to be indicated in cells which blackened with osmic acid. This is a criterion of very doubtful value since blackening with osmic acid may indicate various things, even secretory exhaustion and degeneration as in the Langendorff cells of the thyroid (Severinghaus, '33 a). Urasov ('27) stated that in the acidophiles of the pregnant mouse hypophysis the apparatus is polarized toward the capillary lumen, as it is in that of the guinea pig (Kirkman, '37). Severinghaus ('33), studying the non-pregnant rat, not only was unable to confirm Urasov, but on the contrary found the polarization more frequently away from the capillary. Kull ('25) found the Golgi apparatus to be bipolar in the chromaffine (argen-taffine) cells of the small intestine and regarded the subnuclear portion as being indicative of an endocrine function, but was

unable to explain the significance of the supranuclear portion. Ludford ('25) saw a connective tissue influence in the polarization of the Golgi apparatus in epithelial cells.

Recorded instances of reversal of the polarity of the apparatus are not hard to find (Golgi, '09; D'Agata, '11; Basile, '14; Cajal, '15; Cowdry, '22; Jasswoin, '25, '25 a; Timofejev, '25; Bowen, '26; Cramer and Ludford, '26 a; Henneguy, '26; Nassonov, '27; Litwer, '28; Ludford and Cramer, '28; Weatherford, '29; Ingram, '30; Beams and King, '33; Elefthériou, '36).

Cowdry's ('22) description (fig. 7) of the occasional reversed polarity of the Golgi apparatus in the thyroid cells of the guinea pig was accompanied by the important suggestion that the position of the apparatus probably indicates the direction of secretion, i.e., toward the follicular lumen or toward the blood stream. His evidence in support of this hypothesis, though not extensive, was sufficiently convincing to arouse the expectation that it would help solve the mystery of the Golgi apparatus, but the validity of Cowdry's conclusions was denied by many investigators and Bowen ('26), finding a reversal of polarity of the apparatus in the epithelial cells of the epididymis and the vas deferens, questioned its validity as an indicator of secretory polarity. Giroud ('26, '28), Turchini ('27), Okkels ('32, '34, '34 a) and Elefthériou ('36) declared that the position of the Golgi apparatus is not dependent on the direction of cellular secretion but is determined mechanically and Hirschlerowa ('27) found no reversal of the apparatus in the hyperactive thyroids of metamorphosing amphibia. Cramer and Ludford ('26 a), on the other hand, described reversal of the apparatus in the thyroid, confirming Cowdry's observation, but were unable to find evidence of a corresponding reversal of secretory polarity. Later ('28), however, they observed reversal of both apparatus and secretion in pathologically hyperactive cells.

Some evidence in support of Cowdry's theory was offered by Shiro-Ishimaru ('26) who found that from 6 to 12 days after partial thyroidectomy in the rabbit the Golgi apparatus

in the remaining cells undergoes enlargement and frequently migrates to the basal pole. Cowdry ('34) cited Okkels ('32) and Thomas ('34) as confirming his original observation of Golgi apparatus reversal in the thyroid, implying that they accepted the theory, but in a series of careful papers, Okkels joined Giroud and Turchini in emphatically refuting the theory. Thomas, as Cowdry pointed out, rather supported the theory by reporting reversal under intense excretory activity, but he is of the opinion that the inversion is in no way connected with the direction of glandular secretion, but indicates prolonged stimulation of the thyroid and may be observed with equal facility in secreting cells. Thomas declared (p. 292) "L'interprétation de Cowdry est donc erronée" and again "Cowdry a bien observé, mais mal interprété."

Gillman ('34, '35) placed himself in a contradictory position by declaring "the views of Cowdry, Cramer and Ludford are untenable" and simultaneously subscribing to those views by stating that "the position of the Golgi apparatus yields information as to the *direction* of flow of the cellular secretion." He denied the occurrence of any true reversal of the apparatus in the normal or hyperactive human thyroid, a denial confirmed for the rat gland by his student Fischborn ('35), but he interpreted his failure to observe reversal as demonstrating a corresponding failure of the human thyroid cell to secrete directly into the blood stream. The conclusions which Gillman drew from his morphological observations become untenable when considered in the light of the prior studies upon activated thyroids described below.

Ingram ('30), Wagschal ('31) and Uhlenhuth ('32), using amphibian thyroids activated by anterior pituitary implantations and injections, failed to confirm Cowdry's theory. The final blow to this theory has been dealt by Severinghaus ('33 a) and by Okkels ('34), both of whom used warm-blooded material, and by Uhlenhuth ('34) and Hellbaum ('36) using cold-blooded material. These investigators succeeded in stimulating thyroids to a remarkable degree of activity by means of anterior pituitary gland injections. Since the iodine

content of such activated thyroids decreases (Loeser, '31; Schockaert and Foster, '32) the activation involves augmented discharge of secretion into the blood stream, but the Golgi apparatus, nevertheless, always remains in the apical position which is characteristic of the normal thyroid cell.

Despite the preponderance of evidence to the contrary, Cowdry ('34) still favors his theory in its original ('22) form, and while recent studies on the thyroid have greatly weakened the theory they have not completely discredited it, for the enamel organ remains one very interesting example of what appears to be a true reversal of position of the apparatus, upon secretory activation of inactive cells (fig. 17). In the inactive ameloblasts the Golgi apparatus retains the basal position characteristic of it in cells of the oral epithelium, but becomes apical in cells which become active in the production of enamel (Jasswoin, '25; Beams and King, '33).

While Cowdry interpreted reversal of the polarity of the apparatus as indicating an alteration in the direction of secretion, Litwer ('28) found it to indicate a change in function. In the white mouse he found that when the entodermal cells of the yolk sac are engaged in the resorption of fat the Golgi apparatus occupies a subnuclear position (fig. 12) and holds the smallest droplets within its meshes. As resorption gives way to the secretory phase of the cycle the apparatus shifts to a supranuclear position and the secretion granules appear within its interstices (fig. 11). Jacobs ('27) believed in the secretory significance of Golgi apparatus polarity, but he felt that the position of the apparatus relative to the cell nucleus has been overemphasized to the neglect of the relation between the apparatus and the active cell surface. The work of Litwer suggests the probable soundness of this criticism.

5. ORIGIN OF THE GOLGI APPARATUS

If we are to think of the Golgi apparatus as an integral part of cytoplasmic structure, and not as a mere inclusion, a knowledge of its ontogenetic history becomes of the utmost importance. It should be followed not only through the secretory, or other metabolic, cycle of more or less differentiated

cells, but also through the genesis and differentiation of those cells in the embryo. It, or its homologue, should be traced through all the stages of gametogenesis, and recognized in the fertilized egg (Wilson, '25 a, '26). It should be found throughout cleavage, formation of germ layers, histogenesis and organogenesis. Related to this general problem is the matter of alterations in the volume of the apparatus in glandular cells, fluctuations which in turn involve the questions of transformation into secretory products, restitution, nuclear origin, origin *de novo*, relation to mitochondria, cellular lipoids and vacuome, and behavior during mitosis.

There is a voluminous literature concerning the Golgi apparatus in germ cells. The apparatus was demonstrated in primordial germ cells as long ago as 1914, by von Berenberg-Gossler. Until recently, however, its existence in spermatogonia and oogonia had remained somewhat doubtful, particularly in vertebrates. The majority of investigators in describing the Golgi apparatus during spermatogenesis or oogenesis either mention that this organelle was observed first in spermatocytes or completely ignore the question of gonial Golgi material. Nevertheless a sufficient number of observations of the apparatus in these early germ cells has now been made to make it very improbable that Golgi material is formed *de novo* in the spermatocyte or oocyte. The idiozome was described in spermatogonia of the scorpion by Wilson ('25 a). Nath ('30) reported that even in the unstained, living condition the apparatus may be identified in the earliest oogonia of the earthworm. Golgi rods were described in spermatogonia of *Discoglossus* by Gatenby ('31 b) and in secondary spermatogonia by Hickman ('31). Nester ('32) convincingly described and pictured the apparatus throughout the complete cycle of spermatogenesis, from germinal epithelium to mature sperm, in a pseudoscorpion. The work of Sembrat ('30) included a description of Golgi material in primordial spermatogonia of *Dendrocoelum lacteum* and Gresson ('36) described the apparatus in the spermatogonia of certain insects.

While observations are not so numerous in vertebrate material they are equally convincing. The early discovery of the apparatus in the spermatogonia of Triton by Nassonov ('23) has now been repeated in human material by Gatenby and Beams ('35). These two cytologists illustrated Golgi material in all cell types present in germinal epithelium of the human testis and described the progressive changes which the material undergoes during the maturation process. In view of the added weight given the earlier observations by the work of two such trustworthy observers as Gatenby and Beams, it can no longer be said that the Golgi apparatus is formed *de novo* at any particular stage of germ cell maturation.

Nester's work is of further interest in connection with bi-parental inheritance. In the great majority of animals the Golgi remnant, left after the secretion of the acrosome, moves back through the cytoplasm and is cast out of the sperm, so that, as stated by Bowen ('22): "The Golgi apparatus and idiosome are thus lost from the completed sperm, in which they are represented only by their differentiation product, the acrosome." Nester described a prominent spiral thread around the nucleus of the mature sperm, and traced the origin of this thread from the Golgi apparatus. It would be of great interest to know the fate of the thread after fertilization, for no direct evidence indicating a paternal contribution to the Golgi apparatus of the fertilized egg has yet been presented. Weigl ('12), Gatenby ('19, '31 b), Gatenby and Woodger ('21), Adamstone and Card ('34) and Crabb ('35) also expressed the opinion that in certain instances a part of the Golgi material is left behind as a permanent contribution to the sperm. After discussing Golgi apparatus and mitochondria in relation to bi-parental inheritance Gatenby ('19) stated that "there is now strong probability that the ripe sperm in some forms contains, in addition to nucleus, not only mitochondria but Golgi apparatus, and it seems likely that in cases both categories of inclusions are carried over to the egg." To this meager evidence may be added Weier's descriptions of remarkable similarities between animal Golgi zone and

plant plastid, since numerous studies assign to the plastid a role in heredity. The unconfirmed theory of Bounoure ('31) that the Golgi apparatus (?) is a germ cell determinant should also be mentioned here.

It has been claimed (Bhattacharya, '25, '29, '30, '31; Bhattacharya and Lal, '29; Bhattacharya, Das and Dutta, '29; Narain, '30; Lal, '33) that in certain inframammalian vertebrates the Golgi apparatus of the ovum and the oocyte receives a contribution from the cells of the follicular epithelium through a process of direct infiltration, but verification of such surprising observations is needed.

Our knowledge of the Golgi apparatus in young embryonic cells is very inadequate, although one suspects as probable the following statement from Cowdry ('24): "Some representative of a Golgi apparatus is to be seen in egg cells and in all the cells of developing embryos." While the behavior of the apparatus during histogenesis and organogenesis has been described by several investigators, its existence in the vertebrate embryo during segmentation and germ layer formation is uncertain at the present time. In important studies Hirschler ('18) and Gatenby ('17-'19), using molluscan material, successfully traced Golgi bodies from the egg to the germ-layer stage; Parat ('28) did the same for the vacuome, but Nihoul ('25), working with rabbit material, is the only one to confirm these studies in the vertebrate. Both Fañanás ('12) and Cajal ('15) described the apparatus in various cells of the chick embryo, but only after the germ layers were fully formed.

The chief reason for this serious gap in our knowledge appears to be the old difficulty of distinguishing between Golgi apparatus and mitochondria; a difficulty particularly hard to overcome because of the morphological similarity of the two cytoplasmic structures to each other and to lipoids in young cells. However, Cajal, as early as 1915, considered this problem already solved: "El aparato reticular de Golgi es factor anatomico constante del protoplasma de todas las celulas vivas, así embrionarias como adultas, separandose perfectamente por sus propiedades quimicas, forma y ovolución de las

mitochondrias y demás organitos integrantes de la celula," and the success with which this differentiation has been accomplished in germ cells suggests that a new attempt might prove successful.

Although a *de novo* origin has been advocated by von Bergen ('04) and others for the Golgi apparatus, there is little to favor such a view. One often finds small osmiophilic granules of uncertain significance, in Kolatchev sections, but they are present in addition to the Golgi apparatus and appear to bear no relation to the latter structure. Harvey ('25), finding such granules in the egg cells of the earthworm, interpreted them as indicating the origin of Golgi material from the cytoplasm, but this interpretation has not been adopted by other workers. It is now a matter of common knowledge that in many secretory cells the Golgi apparatus fragments and moves toward the lumen together with the secretory products. In fact Brambell ('25) claimed that in the ciliated epithelial cells of the oviducal glands of the fowl the fragmented Golgi apparatus is extruded into the lumen and a fresh apparatus appears at the base of the cell. Although Brambell's claim has not been disproved, the known differences in staining capacity exhibited by the Golgi apparatus during different phases of cellular activity justify the supposition that perhaps the 'new' apparatus really arises from part of the original Golgi substance remaining in a relatively non-reducing state in the base of the cell. This may prove to be the explanation of the apparent disappearance and subsequent reappearance of the apparatus as described in cells of thyroid and kidney immediately following stimulation (Okkels, '34, '34 a). Ludford's ('25) suggestion that the Golgi material may arise *de novo* in the epididymis was denied by Bowen ('26 a). In a recent paper MacLennan ('36) suggested a *de novo* origin for Golgi bodies, for in certain protozoa he was unable to identify the bodies in late stages of encystment. He found them reforming during feeding stages in ciliates. von Fieandt and Saxén ('36), also, believed that the apparatus is formed *de novo* in certain cells of the internal ear of mammals. The

final answer to this question must await improved technical methods.

Nuclear origin of the Golgi apparatus was suggested by Goldschmidt as early as 1904. Among the few advocates of this very unorthodox view was Honda ('29) who believed that Golgi apparatus in the submaxillary gland originates from the nucleus, being formed by chromatic bodies discharged from the nucleus during 'physiological degeneration' of the cell. Related to this concept is that of Kurashige ('30) who believed that the Golgi apparatus of certain amphibian and reptilian erythrocytes is formed from particles which transude through the nuclear membrane. A similar origin was suggested by certain observations of Saguchi ('28) and Hirschler ('29), according to whom all the Golgi bodies in the young spermatid of a certain Hemipteran fuse to form a single acroblast, following which new secondary Golgi bodies are formed from nuclear sap. However, Gatenby ('31) threw considerable doubt upon the accuracy of this observation. The suggestion of Laguesse ('24) of a possible evolution of Golgi bodies from a paranucleus suggests a mitochondrial rather than a nuclear origin.

The opinion that the Golgi apparatus is the product of certain chemical reactions occurring in cells, and should therefore not be considered as a true organelle, belongs in the same category as a *de novo* origin. If it can be demonstrated clearly that the Golgi apparatus may arise in the cytoplasm independently of any preformed Golgi material, then the former opinion, as expressed by Guthrie ('25, 26), Kurashige ('30), Tennent, Gardiner and Smith ('31), Nahm ('33), Winters ('33) and others, may be considered more favorably than present knowledge warrants, in spite of the fact that in several instances the structures described as Golgi elements by these writers are clearly dissimilar to the classical Golgi apparatus of other investigators.

The belief suggested as recently as 1934 by Chambers, that the Golgi apparatus does not exist as such in the living cell, but is formed by the running together and fusion of vacuoles,

is an old one, having been advanced as early as 1903 and 1904 by Misch and von Bergen respectively. Interest in it was greatly stimulated in 1924 by three papers in which Parat and Painlevé announced their vacuome hypothesis. The theory was then developed and defended in a veritable flood of papers by Parat, Guilliermond, Guilliermond and Mangenot, Covell and Scott, Feyel, Cowdry and Scott, Hibbard, Zweibaum and Elkner, Urtubey and many others. These papers may be found in the bibliographies of reviews by Bowen ('27 a), Guilliermond ('27, '35), Jacobs ('27), Parat ('28), Hirschler ('28), MacBride and Hewer ('31), Gatenby ('31 a) and Voinov ('34). The vacuome hypothesis has been progressively discredited since 1929, largely through the work of Avel ('25), Bowen ('27 a, '28 a), Chlopin ('27), Krjukowa ('29), Beams and Goldsmith ('30), Beams and King ('32, '32 a, '32 b) and Gatenby ('31 a) and recent cytological investigations with the aid of the ultracentrifuge (Beams and King, '34, '35; Beams, Gatenby, Muliyl, '36; Muliyl, '35) have disproved this hypothesis, for at the tremendous speed developed by the ultracentrifuge separate stratification of Golgi apparatus, vacuome and mitochondria occurs. The papers of Gatenby ('31 a) and MacBride and Hewer ('31) give careful analyses of the evolution of the vacuome theory.

Barton-Wright ('31) agreed with Scott ('29) that the Golgi reticulum described by the latter in root tips of *Vicia faba* is canalicular in nature and owes its origin to "the accumulation of water from the condensation of amino-acids during protein synthesis." This conclusion depended upon the observation that a colorless canalicular system could be seen ramifying through the cytoplasm of primary rootlets, while in the secondary rootlets it appeared to be filled with osmium reducing substances. Scott did not look for osmiophilic platelets in her material, however, and there is no doubt that the Golgi reticulum which she illustrated is not the same as the Golgi platelets described by other authors in similar material. On the other hand, it has not been universally acknowledged that the osmiophilic platelets really do correspond to the Golgi zone of animal cells. In addition to the adherents of the vacuome

hypothesis, Weier is an outstanding opponent of the platelet-Golgi-zone homology.

A reviewer of the literature on the Golgi apparatus finds himself returning frequently to male germ cells, for it is here that the apparatus is most readily demonstrated, and because it was here that the first clue to the function of the apparatus was discovered. The typical spermatocytic Golgi apparatus exists in the form of several discrete rods or batonnettes (dictyosomes) arranged around the periphery of the idiozome (fig. 4A). Since these dictyosomes, like mitochondria, may be stained vitally with Janus green, many investigators consider the Golgi apparatus as a part of the general chondriome or as a mitochondrial derivative. Benda (1899) was the first to make this claim, because in *Helix*, both mitochondria and Golgi batonnettes stained violet by his alizarin-crystal-violet-mitochondrial method. He therefore assumed the batonnettes to be metamorphosed mitochondria. This claim was developed extensively by Fauré-Fremiet ('10). Other cytologists who held more or less similar views, derived from a study of invertebrate nerve cells, were Monti ('15), Ross ('22), Miglia-
vacca ('30) and Hosselet ('29, '29 a). However, their interpretations were flatly contradicted by Beams and King ('32) who found no evidence whatever for a mitochondrial origin of the Golgi apparatus in the nerve cells of grasshoppers. Parat and his collaborators devoted much attention to the task of bringing the Golgi dictyosomes and the mitochondria together in a single category. Parat ('28, '29), described the metamorphosis of mitochondria into Golgi bodies in spermatocytes of *Discoglossus*. But Gatenby ('31 c) pointed out that other workers, including himself, had already described the Golgi apparatus not only in spermatocytes but also in spermatogonia and primordial germ cells of *Sauropsida*, which implies that the Golgi bodies cannot be newly formed in the spermatocytes. Investigators who still believe in a mitochondrial origin of the Golgi apparatus are Ma, Lim and Liu ('27), Ling, Liu and Lim ('28), Ma ('28, '28 a), Koehring ('30) and Hosselet ('31). In elaborating his theory of fields of restitution with reference to irreversible secretory processes, Hirsch

('31) quoted the following from Ma's ('28 a) work on secretion: "Elaboration of secretion continues until the mitochondria present are 'converted' into zymogen granules; the liberated FL (fuchsinophile property and Golgi-lipoid component) tends to collect within the intermediate zone as the luminal zone becomes filled with zymogen granules and gives rise to the compact form of the Golgi apparatus." Hirsch added that "this connection between mitochondria and Golgi apparatus is suggested by bleaching osmic preparations and restaining with fuchsin-methyl-green; the bleached lipoid reacts like the mitochondria." If the relation between mitochondria and Golgi apparatus is dependent upon the truth of this observation it warrants no discussion, for it is well known to cytologists that the Golgi apparatus in a bleached osmic preparation does not stain with acid fuchsin, although it is thus illustrated by Ma, and has the confirmatory interpretations of Hirsch. Koehring, after making a general survey of the reaction of neutral red upon numerous living animal organisms, concluded that "the nature of the lipoidal Golgi structure usually intimately related to the vacuolar system, of parabasal bodies and other modifications of similar substance suggests the adaptability of but one fundamental cytoplasmic inclusion . . . the mitochondrial complex."

A still different opinion was voiced by Johnson ('31) who did not regard the dictyosomes as homologous with either mitochondria or Golgi apparatus. Johnson, however, receives but little support from other investigators in this belief.

In general, most workers in this field will agree with Beams and King ('32) who wrote:

As regards the suggestion brought forward by Parat that the mitochondria hypertrophy and give rise to the Golgi bodies we cannot agree. We have found no evidence that could possibly be interpreted to support Parat's conception, and while it may very well be assumed that the mitochondria and Golgi bodies are closely related, we are convinced from our studies that the proposed relationship figured and described by Parat, Hosselet, and Radu does not represent the actual condition of affairs.

The correctness of this statement seems evident from abundant confirmation in the repeated observation that there is a differential stratification of mitochondria and Golgi apparatus in ultracentrifuged cells (Beams and King, '34, '35; Muliyl, '35; Beams, Gatenby and Muliyl, '36; Dornfeld, '36).

If the Golgi apparatus is a permanent structure in the cell, it is apparent that there must be some mechanism insuring the redistribution of Golgi material to each daughter cell resulting from mitosis and if the Golgi substance plays a part in the carrying of hereditary factors, we should expect such a redistribution to consist in as precise and equal a division of the original material as occurs in the redistribution of chromatin. On the other hand, if the importance of the apparatus in cellular activities is of qualitative rather than quantitative character, it should not be confusing to find that this redistribution is indefinite rather than exact in nature. The studies of Hirschler, Gatenby, Bowen, Wilson, and many others have shown that during mitosis the division of the Golgi apparatus, though clearly not a mechanical result of cleavage, is only approximately equal. The different ways in which this division occurs were discussed by Wilson ('25, '25 a).

Although the behavior of the Golgi material during cell division cannot be compared with karyokinesis in respect to exactness of division, the fact remains that it seems, as Wilson ('25) has said, a phenomenon of analogous type. The following passage from Bowen ('26 a) summarizes contemporary opinion concerning this subject:

In cell division it is very frequently divided by processes of astonishing complexity and regularity, which make certain a physical continuity of substance from one cell to another. Furthermore, it is generally agreed that the Golgi apparatus is capable of growth, and several observers have described the apparent multiplication of Golgi bodies by a process of fission during the vegetative phase of cell life. . . . the division of the Golgi material always partakes of an essentially mass division, and the impression which one gets both in mitosis and in the resting cell is that of a fragmentation

process. Thus there is every reason to believe, as Wilson has suggested, that the Golgi apparatus is a substance relatively devoid of specific organization into units of differing significance. It is merely a mass of material which is capable of maintaining itself and of reproducing itself by processes of mass division which are in essence based upon mere fragmentation and have nothing further in common with the highly precise divisions of nuclear chromatin.

In amitotic cell division, as described by Gatenby and Hill ('34) in tissue cultures of *Helix*, the Golgi apparatus is said to go over entirely into one daughter cell, leaving the other cell without any demonstrable Golgi material.

EMBRYONIC DEVELOPMENT OF THE RESPIRATORY PORTION OF THE PIG'S LUNG ¹

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THREE PLATES (SIX FIGURES)

The question of the presence or absence of a histological epithelium lining the respiratory portion of the mammalian lung cannot be settled until the changes which occur during the transition from late fetal to postnatal stages are thoroughly studied. It is not necessary to review here the controversial literature on the embryonic and postnatal mammalian lung as this has been adequately treated in the works of Fried ('34), Bremer ('35) and Bargmann ('36). The present study, based on close, successive stages of very fresh fetal and postnatal pig lungs, deals with the formation of the respiratory spaces and the fate of the connective tissue and the epithelium of the terminals of the bronchial tree. Particular attention has been paid to the arrangement of the blood capillaries in the developing respiratory portion of the lung.

MATERIALS AND METHODS

Fetal pigs from sixty-one litters were secured at Cudahy's abattoir, Omaha, so that the 130 fetal and three postnatal pigs prepared represented thirty fetal stages based on millimeter crown-rump length, and two postnatal stages, as follows (number of litters in parentheses): (1) 55, (1) 70, (2) 75, (1) 80, (1) 90, (1) 115, (2) 120, (1) 130, (2) 140, (3) 155, (1) 165, (5) 170, (1) 175, (1) 180, (2) 185, (1) 190,

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(2) 195, (5) 200, (1) 210, (1) 215, (4) 220, (4) 230, (1) 235, (4) 240, (1) 250, (3) 255, (2) 260, (1) 270, (1) 275, and (3) 290 mm., the fetal length at term. One postnatal specimen was six and two (one litter) were 18 hours old. Stages 200 to 240 mm. proved critical in development of the respiratory portion.

The fetal lungs were fixed in Zenker-formol within 5 minutes after removal of the uterus from the sow, and about 30 minutes after the sow was killed. In many cases the fetal heart was beating vigorously when exposed. The lungs, trachea, heart and larger blood vessels were always removed and placed in the fixative together to avoid mechanical injury.

Where there were four pigs in a litter, in one the lung was sliced at 1-cm. intervals with a thin razor blade before it was removed from the chest cavity. In a second the fixative was injected via the trachea at low pressure before the thoracic cavity was opened. In the third, when the heart was still beating the pulmonary veins were clamped and the heart allowed to contract several times to force more blood into the lung before removal. In the fourth, the lung was removed without previous treatment.

The tissues were embedded in nitrocellulose and sectioned at 13 μ . The sections were mounted in series, usually four sections to the slide and eight slides to the series, by the use of oil of cloves, after which the nitrocellulose was removed in ether-alcohol. To keep the sections uniform, the sections for a slide were lined up on the knife, well moistened with 75% alcohol, and then removed from the knife with thin paper and stored moist with 75% alcohol in Petri dishes until convenient to mount them. In mounting, each paper was placed, with the sections up, on a pad of smooth filter paper, and the albumenized slide pressed down on the sections so that they adhered to the slide when the paper was removed. After blotting to remove excess alcohol and to flatten the sections, clove oil was poured on them.

Heidenhain's iron hematoxylin, phosphotungstic acid hematoxylin, hematoxylin-eosin-azure II, Mallory-azan, Foot's

modification of Bielschowsky's silver impregnation, and orcein were used in each series. Both Mallory-azan and acridin red were used as counterstains after the Foot stain. Methyl green was used over the orcein.

In two of the 290-mm. and one 18-hour postnatal stage, 3% silver nitrate was injected by the trachea, and allowed to stand 15 to 20 minutes. Then the lung was immersed in 3% silver nitrate 3 hours, then in 10% formalin 24 hours, washed in water, dehydrated and embedded. After sectioning and mounting the silver was further reduced on the slide with Eastman Kodak Company D-73 developer, and the sections counterstained with Mallory-azan, hematoxylin-eosin-azure II, phosphotungstic acid hematoxylin and Harris' hematoxylin.

OBSERVATIONS

The pre-critical stages (55 mm., 115 mm. and 160 mm.)

At 55 mm. a section from a lung shows indefinite septa which divide the lobe into primordial lobules. The largest two or three orders of primordial tubules are lined with folded tall columnar epithelium. From these the remaining two or three orders branch and appear in each lobule as tubules with columnar epithelium and dilated ends, most of which branch dichotomously. The nuclei lie at the base of the epithelial cells or next to the lumen with equal frequency. Mitotic figures appear in all tubes, but more frequently in the smallest ones and their terminals. Cilia are not seen.

The connective tissue is condensed around the tubes and at their dilated ends where it is wider but less dense than along the sides. The wide spaces between the dense connective tissue are filled with loose connective tissue. The blood vessels and capillaries are more prominent in the loose connective tissue, but occasionally a capillary invades the dense connective tissue around the tubes. The reticular fibers form a loose network in the loose connective tissue and a more compact one in the denser tissue around the tubes, while at the base of the epithelium they form a very dense layer. At the terminals of the tubes the fibers of this layer are widely

scattered and arranged in roughly parallel semi-circles about the ends. As the growing terminal bud pushes into the connective tissue, these fibers form the condensed layer of reticular fibers at the base of the epithelium of the tubule.

At 115 mm. the number of primordial lobules has increased about fivefold as seen in sections without the aid of reconstruction. Around the largest tubes is a varying thickness of connective tissue continuous with that of the interlobular septa. The largest of these tubes are lined with folded pseudo-stratified columnar epithelium. The others, lined with tall columnar epithelium, give rise to four or five orders of mostly dichotomously branching tubes; a single section of a lobule presents twenty to thirty such tubules cut at various angles, and lined with columnar epithelium. The nuclei in the smaller tubules are chiefly at the apices of the cells. Mitoses occur in the epithelium as at 55 mm., and are seen often in the connective tissue cells. As the lung grows and the number of tubules increases, the area of loose connective tissue appears reduced in section so that now its width equals that of the dense tissue around the tubes. Cilia cannot be seen even in the largest tubes.

Capillaries are abundant in the connective tissue around the tubes and at their terminals where they occasionally extend through the condensed layer of reticular fibers to the base of the epithelium. Some capillaries even run between these fibers and the base of the epithelium.

At this stage, the reticular fibers show no change in distribution, although with the increase in the number of tubes they appear heavier. Some collagenous connective tissue and smooth muscle is being formed around the largest tubes and blood vessels, but around the smaller tubes the condensed layer of connective tissue contains only reticular fibers.

At 160 mm. a section from a lobe has roughly twice as many lobules as at 115 mm., but the lobules are smaller and the lobe, although larger, has not doubled in size. Each lobule in a given section contains two or three fairly large tubes lined with somewhat folded, tall columnar epithelium. From

these arise three or four orders of dichotomously branching smaller tubules lined with columnar epithelium. Some of the small tubules are lined with cuboidal epithelium. From the ends and sides of these tubes arise a large number of epithelial buds which appear as spherical, acinar masses of cells with little or no lumen connecting with that of the original tube, except at the periphery of the lobule where the buds are more open. In cross sections they appear as circles of cells in the dense connective tissue. Mitoses of epithelial cells are fairly frequent, especially in the small tubules. Cilia are seen in the large primitive bronchi, but not in any tubes in the lobules.

The increasing number of tubes and end buds are separated by dense connective tissue except for small stretches of loose connective tissue in the wider intervals. There are more collagenous fibers around the larger tubes and blood vessels, and in the interlobular septa.

The critical stage (200 to 220 mm.)

At 200 mm. the tubular complex in its progressive branching and growth into the connective tissue shows changes which forecast the definitive pulmonary structure of tubular air conductors and a respiratory portion. At this stage in development, the continuous epithelial lining of the tubules begins to become discontinuous and it accordingly might be called the critical stage. The epithelium in more of the largest tubes in the lobule appears pseudostratified (fig. 1, B) while the small tubules are lined with cuboidal epithelium, except in their end buds where it is columnar. Not far from the origin of the third or fourth order of tubes in the lobule, the epithelium may become cuboidal and later become discontinuous and leave the tubular wall bare of epithelium, except for occasional cells, isolated or in groups of two to four (fig. 2, I). The cuboidal and columnar cells in the end buds (figs. 1 to 5, EB), especially at the lobular periphery, persist long after the epithelium has disappeared from the stretches just proximal to them.

At 200 mm., a section from a lobe is considerably larger than at 160 mm. and shows a small increase in number of lobules, each of which contains three or four orders of tubes lined with pseudostratified or columnar epithelium, and a terminal order lined with cuboidal epithelium. The latter occasionally is discontinuous, especially around the angles where branches arise, so that a capillary or the underlying connective tissue is exposed to the lumen (figs. 1 and 2, EC). These tubules end with dividing epithelial buds, lined with columnar or cuboidal cells.

Mitoses are fairly frequent in the epithelium of the end buds. They appear less often than at 160 mm. in the lobular tubes, and gradually diminish toward the largest primordial bronchi, where they are few.

Practically all the connective tissue is dense between the tubules and their closely packed end buds. Capillaries are found more frequently than at 160 mm. among the reticular fibers at the base of the epithelium. There is the same relation of reticular fibers as at 160 mm. Collagenous connective tissue is more pronounced around the larger tubes and blood vessels.

Later stages (220 to 290 mm.)

At 220 to 230 mm. the number of lobules in a section of a lobe has increased somewhat over that at 200 mm. The lobules on the average are larger, and the lobe as a whole is considerably larger. In each lobule eight to twelve orders of branches are counted. The peripheral tubes are now irregularly elongated and expanded and have branches and subbranches which end in wide sacs formed by the dividing end buds (fig. 2, S). Along the walls of these spaces proximal to the end buds much of the epithelium is absent, and capillaries and connective tissue are exposed to the lumen. As the distance increases between the ends of the epithelial lined tubules and the lobular boundary (figs. 1 to 5, B to P), the spaces between them become longer and assume somewhat the appearance of alveolar ducts as seen after birth. The epithelium is columnar to cuboidal in most of the end buds and

mitoses are fairly frequent; in some end buds the epithelium is incomplete. Mitoses are somewhat less frequent in the tubules than at 200 mm. and appear even less frequently in the largest primordial bronchi.

All of the connective tissue is now dense and is greatly reduced in amount between the enlarged, non-epithelial lined spaces and the great number of end buds at their terminals. These buds are 30 to 40 μ in diameter.

Along the walls of those spaces where the epithelium no longer forms a continuous membrane, the epithelial cells, singly or in small groups, are often shrunk and contain pycnotic nuclei. In places the lumen of such spaces is bounded by connective tissue cells partially incorporated in the condensed reticular fibers and the accompanying ground substance. Occasionally large flaky cells with small nuclei appear in the spaces; these are probably desquamated cells from the amnion or epidermis.

Capillaries are more frequent than at 200 mm. among the condensed reticular fibers lining the above described spaces and the end buds. Frequently they bulge into the lumen as loops, or run for short distances as naked capillaries along its wall. In these cases no membrane is seen between the red blood cells and the lumen except the endothelium with its nuclei. Where the capillaries touch the bases of epithelial cells, these separate and the capillaries become exposed to the future air passages. Occasionally a shrunk epithelial cell is seen over a capillary, or at the angle of its emergence through the reticular fibers. Where the continuous epithelium of a tubule ends a capillary often extends through the dense reticular fibers to be exposed as a loop in the future air space (fig. 3, EC'). In looking at these preparations, it is difficult to escape the conclusion that the capillaries are actively pushing through the connective tissue and the epithelium. In any event the sharp separation of epithelium from vascular connective tissue cells no longer holds.

The reticular fibers are increased in number and condensed at the base of the epithelium of the large primordial bronchi.

These fibers gradually decrease in the direction toward the end buds. With the increase in extent of the peripheral epithelium-free spaces and in the number of their end buds, the condensed reticular fibers of two adjacent spaces or of two end buds frequently become confluent and form a common support for the wall of the two spaces or buds. Reticular fibers are seldom seen over the exposed surface of a capillary at this stage, but occasionally are seen along its side. There are a few scattered collagenous fibers in the connective tissue about the finer ramifications of the tubules.

At 250 mm. the lobules are larger than at 220 to 230 mm., and their number is probably somewhat greater. In each lobule are four or five orders of tubules, the largest lined with folded tall columnar and the rest with columnar epithelium, except for a few cuboidal cells at the end of the epithelium of the tubule. From the latter out to the end buds there are now eight to ten orders of branches of epithelium-free spaces suggestive of alveolar ducts, which have widened and lengthened, so that the distance between the ends of the epithelium-lined tubules and the lobular periphery is further increased. The final branches of these 'spaces' end at the lobular periphery, and within the lobule, as expanded fan-shaped sacs formed by the dividing end buds which occupy their walls (fig. 3, S). Most of the end buds are opened as horseshoe-shaped cups, or spread out as wide crescents. A few remain as more or less solid buds. There is a marked thinning of the connective tissue walls between adjacent spaces. With the increase in size and number of the branching spaces the connective tissue is further decreased in amount and begins to appear lace-like. The epithelium of the peripheral spaces is limited chiefly to the end buds. At the lobular periphery the latter occupy much of the room along the connective tissue septum. Only short stretches of epithelial cells, and irregular isolated ones, are occasionally seen in the 'alveolar duct-like spaces.'

Capillaries are more prominent in the lining of the spaces and characteristically loop around the angle of evaginations from them. In many closely adjacent spaces, capillaries wind

through the thin wall to be exposed first to one lumen and then the other. There are more confluent strands of condensed reticular fibers in the thinner connective tissue walls between the duct-like spaces. There is no change in the collagenous fibers. Cilia appear in some large intralobular tubules.

At 275 mm. three or four orders of tubules lined with columnar epithelium are present in each section of a lobule. A few cuboidal cells line the ends of these tubules. The duct-like spaces devoid of a continuous epithelium have elongated greatly and form a close network in the future respiratory area, and the distance between the ends of the epithelium-lined tubules and the lobular periphery is greater than at 250 mm. End buds are now seldom seen except at the subpleural periphery where a few appear as irregular spaces lined in part with epithelial cells (fig. 4, EB). Nearly all of the 'ducts' next to the interlobular septa (not subpleural) are free of histological epithelium.

With the increase in size and number of the alveolar duct-like spaces, the connective tissue now appears as a network of thin strands among the future air spaces although some of the partitions have the thickness of three or four connective tissue cells; the lobule has an increasingly lace-like appearance.

Epithelial cells in the 'ducts' now appear only as occasional, isolated, apparently loosely attached, irregular cells with pycnotic nuclei. These spaces, which have elongated greatly, now look more like alveolar ducts of the postnatal animal, but the lack of alveoli in their walls, and the impression that they are still growing and branching preclude such a designation.

The walls between these 'ducts' contain endothelial cells, a few lymphocytes, and connective tissue cells. When the epithelium is absent, the latter frequently border on future air spaces. This is especially clearly shown in Foot silver preparations counterstained with Mallory-azan. In hematoxylin-eosin-azure II preparations a few of the connective tissue cells in the walls and at the angles of protruding capillaries, are larger than the average, are spherical to irregular

in outline, and their cytoplasm tends to be vacuolated and pinkish. Some of these are deep in the septal wall, but in others half of their bodies protrudes into the future air spaces. They are suggestively like the septal cells of Lang ('25).

There are increased numbers of capillaries exposed to the future air spaces in the walls of the 'ducts' and in their terminals where the epithelium is gone. With the thinning of the walls between adjacent spaces, capillaries wind through these walls exposed first to one potential air space and then another. In other places a septal wall lies between two capillaries, each exposed to its respective air space. Capillary loops dip around the angles of branches from the duct. At the lobular periphery, with the progressive disappearance of epithelium from the terminals, capillaries become exposed in these future air spaces.

Reticular fibers are now seen chiefly as condensed strands partially lining the spaces, or in the middle of thin walls between adjacent ones. With the continued thinning of the walls the latter arrangement becomes much more frequent so that with low magnification this part of the lung appears as a delicate lacy network. Collagenous fibers are thicker and denser around the larger tubes and blood vessels. Cartilage appears only in the largest bronchi.

At 290 mm., the length of the fetal pig at term, the number of epithelium-lined tubes in a section of a lobule is about the same as at 275 mm., but the tall columnar epithelium of the largest ones is more folded, and the columnar epithelium of the rest extends to the cuboidal epithelium of the terminal tubules. The latter have increased in diameter, and end still farther from the periphery of the much-enlarged lobule than at 275 mm. Cilia appear in the largest tubes of the lobule.

Mitoses are rare in the epithelium of a large bronchus, and are limited chiefly to that of the terminal bronchioles where they are seen occasionally. They appear fairly often in the end buds at all stages until they lose their epithelium, as most of them have at the present stage (290 mm.). Although

mitoses occur in the connective tissue at all stages, they are less frequent now.

In a given section two or more potential alveolar ducts arise from each terminal bronchiole. These ducts are more expanded and longer than the similar structures at 275 mm., and their many branches terminate in a closely packed mass of primordial air sacs which are so intermingled that ducts and sacs are separated only by thin connective tissue septa.

The large spaces separated by thin walls give the sectioned lung a lace-like appearance under low magnification. The large irregularly shaped 'septal' cells with vacuolated cytoplasm seen at 275 mm. are more prominent now, and more of them extend part way into the air spaces from the angles of septal walls and capillary loops.

Only occasionally does an epithelial cell appear on the walls of the alveolar ducts, and it is usually at the angle of protrusion of a capillary. A membrane cannot be seen over the endothelium of exposed capillaries. Nearly all the end buds are now potential respiratory spaces and are generally free of an epithelial lining except a few next to the pleura and the interlobular septa. In section these contain isolated epithelial cells or groups of two to four.

With the increase in number of thin connective tissue walls between the spaces, the capillaries become very prominent and exposed to the primordial air space. The epithelium in the terminal spaces loses its continuity where the capillaries penetrate the condensed reticular fibers and reach the base of the epithelium.

The reticular fibers lining adjacent primordial air spaces continue to approach one another and become confluent so that many more septa now have a single layer of condensed reticular fibers at least through part of their extent. These strands of reticular fibers occupy the centers of the septa while the capillaries either penetrate them, or lie on either side exposed to the future air space. Connective tissue cells are scattered among the reticular fibers and capillaries. It will be seen that in the postnatal stages this arrangement of the reticular fibers in the septa becomes the rule.

The condensed layer of reticular fibers is very heavy in the largest bronchi, but grades off through all the orders of branching until that at the base of the cuboidal cells in the terminal bronchiole at term may consist of but five or six fibers in section. These fibers become gradually reduced in number as they continue into the walls of all the potential air spaces, and finally assume the pattern described above. In this way the delicate strands of reticular fibers form a supporting framework for the capillaries and connective tissue cells of the finer air spaces.

Postnatal stages (6 and 18 hours)

In the postnatal stages the pulmonary veins were clamped and the heart allowed to beat a few strokes to fill the capillaries with blood before injecting the fixative at very low pressure by way of the trachea. The respiratory spaces were somewhat distended, but there was no tearing of tissues or extravasation of blood.

There is less folding of the epithelium in the large bronchi than at 290 mm., and many of the smaller bronchioles are lined with low columnar to cuboidal epithelium. There is no apparent change in number of tubes in a lobule. Mitoses appear fairly often in the epithelium of the smaller bronchioles, but rarely in the walls of the finer air spaces.

Presumably due in part to distension by the fixative, and in part to respiration, the septal and alveolar duct walls appear thinner and more openly lace-like than at 290 mm., and the lobules in section are much larger. Also, fewer air spaces appear in a given field (fig. 6). In most of the septa only a few scattered connective tissue cells appear in the rich network of capillaries. 'Septal cells' with vacuolated cytoplasm appear among the capillaries. Some of these show ameboid movement. The cytoplasm of these cells is more prominently vacuolated than in those at 290 mm.

There seem to be few if any epithelial cells or any sort of membrane in any alveolar ducts, although an occasional isolated cell (possibly of epithelial nature) may be present in the

lining of the air spaces next to the subpleural connective tissue (fig. 6). In places the faint ground substance of the connective tissue forms a thin film between the septal walls and the air spaces, but the capillary loops projecting into the air spaces appear bare.

The septal walls consist predominantly of a core of reticular fibers supporting networks of capillaries which wind back and forth through the septa and lie exposed for long stretches to the air spaces on either side.

SUMMARY AND DISCUSSION

In the early stages of development of the pig's lung there is a complete epithelial lining of the bronchial tubes. Later, the smallest tubes ramify and occupy progressively larger volumes of the lung. The epithelium becomes discontinuous so that the capillaries and connective tissue of these tubes are exposed directly to the lumen. Thus arise potential alveolar ducts which in turn will communicate with those areas in the postnatal lung where respiration will take place.

The early break in the epithelium divides the primordial air passages into three zones. With the reservation that it is impossible to homologize these structures exactly with those of the postnatal lung, these might be called 1) bronchi and bronchioles, lined with epithelium; 2) potential alveolar ducts, devoid of epithelium; 3) the end buds, lined with epithelium, which by growth and division irregularly extend the potential conducting and respiratory spaces. At term the epithelium in the end buds is partially or completely absent.

Probably the main factor in the exposure of the vascular connective tissue to the lumen of the duct system is the failure of the epithelium to keep pace with the very rapid extension of the branching terminals toward the lobular periphery. In this process the epithelium of the tubes stretches and breaks into fragments which lose their prominence as the primordial spaces expand.

The growth of the capillaries seems also to be a factor in the breaking and disappearance of the epithelium, as found

in the human fetus by Palmer ('35), for wherever in the future respiratory area a capillary pushes through the condensed reticular fibers and touches the base of the epithelial cells, these break apart, become irregular in shape and loosely attached, and their nuclei often become pycnotic. Although it seems clear that the embryo executes breathing movements for some time before birth, it has not been demonstrated that the aspirated amniotic liquid helps to distend the future respiratory spaces.

Though the epithelium is low cuboidal in places there is no evidence of overlapping phalanges as seen by Stewart ('23) in dogs and rabbits. Flint ('06) studied the development of the lung of the pig. He noted exposed capillaries and thought they were covered by a membrane much like non-nucleated plates. In my material non-nucleated plates were not seen at any stage even with the precarious silver nitrate technique. Silver often impregnates the capillary endothelium where exposed to the air space, and sometimes the outlines of connective tissue cells, as well as epithelial cells. Neither is the fine membrane over the capillaries in various mammals described by Seeman ('28) evident in the pig lung.

The final disposition of the reticular fibers in late fetal and postnatal stages is noteworthy because of their prominence in the septal walls, and because of their confinement chiefly to the center of the wall as a dense layer so that the capillaries lie on either side exposed to the air spaces, or pass through them from one space to the other. Of the scattered cells lying among the capillaries in the septal wall, many are associated closely with the reticular fibers, and probably have the potentiality of forming phagocytes. Frequently, where capillaries do not line the wall, reticular fibers and their ground substance, and connective tissue cells lie exposed to the air space.

The distinctive cell with irregular shape and vacuolated cytoplasm, possibly the septum cell of Lang ('25), needs to be traced in later postnatal stages. As it is followed from

275 mm. into the postnatal stages, it shows increase of vacuolization and depth of staining in the cytoplasm, and more actively ameboid forms. The fact that it is found within the septal wall as well as on its border partly projected into the air space indicates that it is not exclusively of entodermal origin.

CONCLUSIONS

1. In the fetal pig lung the walls of the future respiratory spaces are formed from the branching buds of the tubules which fill most of the room occupied in earlier stages by connective tissue.

2. As the end buds grow, the epithelium of the small tubules behind them becomes discontinuous, and gradually disappears, leaving potential ducts and spaces lined with capillaries and connective tissue.

3. The intertubular connective tissue of the early stages remains as the thin septa between the future air spaces.

4. The capillaries of the septa lie next to the future air space on either side of the walls and pass through them from one side to the other.

5. The reticular fibers continue from beneath the terminal bronchiolar epithelium into the centers of the septa between the potential respiratory spaces.

6. The fact that the epithelium begins to disappear in embryos of 200 mm. and is largely absent before term, indicates that the change from a series of epithelial-lined passages into those in which the epithelium is not apparent is obviously not due to the changes occurring at birth.

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LITERATURE CITED

- BARGMANN, W. 1936 Die Lungenalveole. von Möllendorff's Handbuch der mikr. Anat. des Menschen, Bd. 5, S. 799.
- BREMER, J. L. 1935 Postnatal development of alveoli in the mammalian lung in relation to the problem of the alveolar phagocyte. Carnegie Cont. to Embryol., no. 147, p. 83.

- FLINT, J. M. 1906-1907 The development of lungs in the pig. *Am. J. Anat.*, vol. 6, p. 1.
- FRIED, B. M. 1934 The lungs and the macrophage system. *Arch. Path.*, vol. 17, p. 76.
- LANG, F. J. 1925 The relation of lung tissue to tuberculous infection in vitro. *J. Infect. Dis.*, vol. 37, p. 430.
- PALMER, D. M. 1935 The lung of a human foetus of 170 mm. C. R. length. *Am. J. Anat.*, vol. 58, p. 59.
- SEEMAN, GEORGE 1928 Über den feineren Bau der Lungenalveole.—Beitrage zur Frage des 'Respiratory Epithel.' *Beitr. zur pathologischen Anat. u. allg. Path.*, Bd. 81, S. 508.
- STEWART, F. 1923 An histogenetic study of the respiratory epithelium. *Anat. Rec.*, vol. 25, p. 181.

DESCRIPTION OF PLATES

Figures 1 to 6. Semi-diagrammatic drawings, outlined with camera lucida at table level, from sections of fetal and postnatal pig lungs to show the fate of the epithelium in the developing respiratory spaces. Zeiss 8 mm. apochromatic objective and $\times 15$ ocular. A, alveolus-like pocket; B, branching tubules lined with epithelium; BRB, branching end bud; D, potential alveolar duct; D', alveolar duct; E, epithelium with black nuclei; EB, end bud, EC, exposed capillary, or connective tissue, where the epithelium is absent; EC', exposed capillary at end of epithelium of tubule; I, isolated epithelial cells; M, connective tissue, stippled; P, pleura; R, red blood cells and blood vessels in red; RC, strand of reticular fibers with capillaries on either side exposed to the air spaces; RT, condensed reticular fibers represented by a single black line at the base of the epithelium, and in the walls of the finer spaces; S, terminal expansions of the potential alveolar ducts; SB, side branch.

PLATE 1

EXPLANATION OF FIGURES

- 1 A 200-mm. stage showing branching tubules lined almost completely with epithelium, which is flattened in places, and interrupted occasionally. A capillary is exposed at EC. Note the relatively large amount of vascular connective tissue.
- 2 A 220-mm. stage. Epithelium-lined tubules, B. Potential alveolar ducts, D, with the epithelium partially absent appear between the tubules and the proliferating end buds.

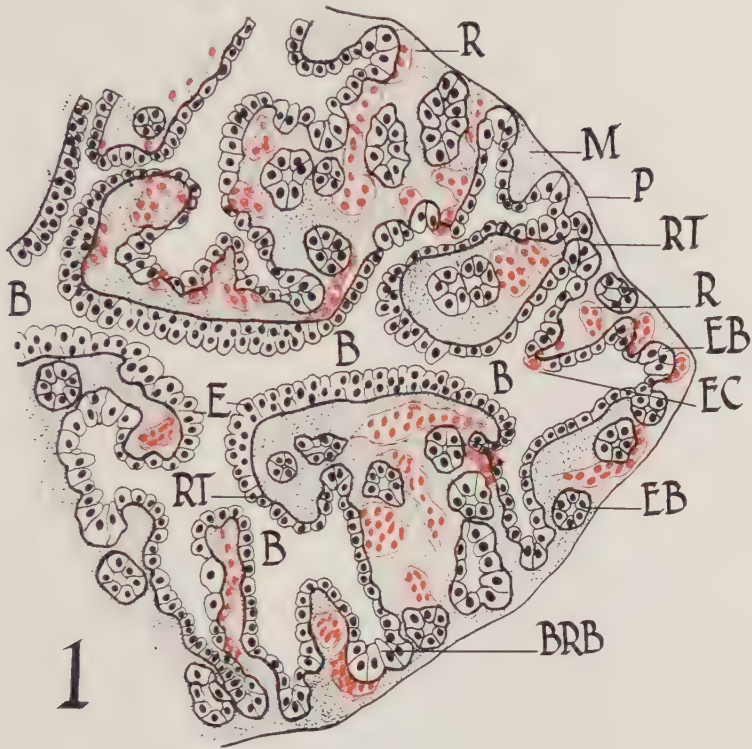


PLATE 2

EXPLANATION OF FIGURES

3 A 250-mm. stage. The epithelial-lined tube, B, branches and continues into several potential alveolar ducts, D, which lack a continuous epithelium. The terminal expansions of the ducts, S, are wider and more numerous than at 220 mm.

4 A 275-mm. stage. Little epithelium appears in the potential alveolar ducts, and most of that in the end buds is gone.

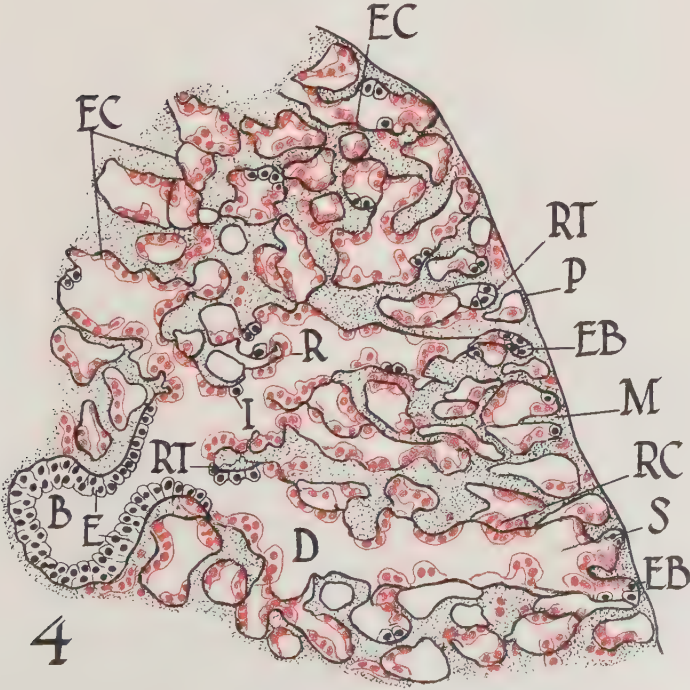
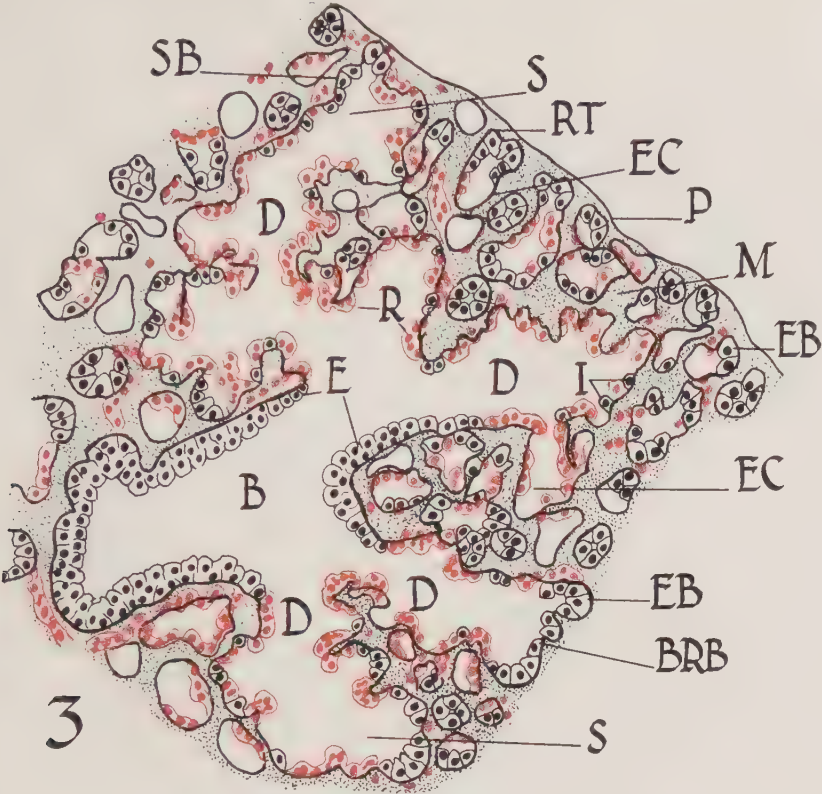
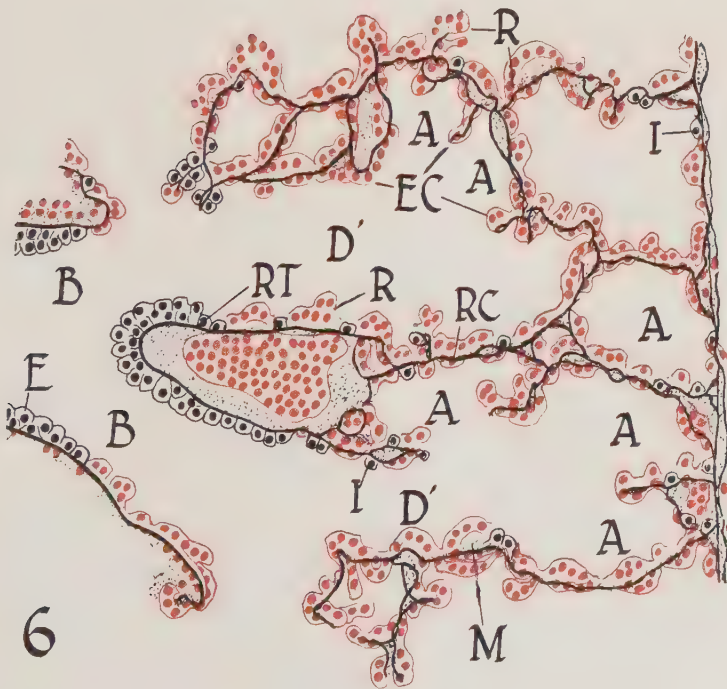


PLATE 3

EXPLANATION OF FIGURES

5 A 290 mm. (term). The alveolar ducts are further expanded so that the connective tissue between the spaces is much reduced. The epithelium is practically gone from the ducts and the capillaries and connective tissue are exposed to the air spaces. The epithelium is absent from most of the end buds, which are now potential respiratory spaces. The reticular fiber layers of adjacent spaces frequently become confluent as at RC.

6 Eighteen-hour postnatal stage. The respiratory spaces are much expanded by breathing and by the injected fixative, so that their number in the field is reduced. The capillaries lie exposed to the air spaces on either side of the reticular fibers in the septal walls. The connective tissue, M, is much reduced.



CONJOINED TWINS AND TRIPLETS IN TROUT

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ONE PLATE (TWO FIGURES)

Isolated instances of the occurrence of double individuals among fishes have been frequently reported since the time when Aldrovandi first published a figure of such an anomaly in his 'Monstrorum Historia' (1642). Most of these reports concern embryos still adherent to the yolk sac and the survival of such individuals beyond the yolk sac stage seems rare. Extensive bibliographies of the subject of conjoined fishes are to be found in the papers of Schmitt ('01), Gemmill ('12), and Stockard ('21); in Newman's book on 'The Physiology of Twinning' ('23); and in Dean's 'Bibliography of Fishes' ('16) and therefore no attempt will be made to completely review the voluminous literature of the subject in the present account. It will suffice to point out that the great majority of the contributions are merely more or less detailed descriptions of one or two double embryos culled from the fry in fish hatcheries; only a few workers have been able to study any considerable series of specimens.

Windle (1895) presents an analysis of forty-six trout embryos showing various degrees of doubling ranging from those in which there are two anterior ends united at the eye level, the rest of the body being single, to those in which there are two quite complete embryos joined only by their attachment to a single yolk sac. Schmitt ('01) describes the details of the internal anatomy of various types of double embryos in an investigation based on about 400 conjoined twins of *Trutta lacustris*, *Trutta fario*, *Trutta salar* and *Salmo salvelinus* from hatcheries in the neighborhood of Würzburg,

Germany. Gemmill in his monograph on 'The Teratology of Fishes' ('12) ably reviews the literature on the subject and analyzes the results of his own investigations on material derived mostly from the hatcheries at Lochwinnoch and Loch Lomond. This material consisted of seventy-one twin embryos and one triple individual. Complete accounts of the anatomy of the various types, based on a study of serial sections, are given. Stockard ('21) presents an explanation of the origin of these double individuals based upon his studies on the experimental retardation of developmental rate in *Fundulus* and makes some observations on the proportions of the various types as they occur in collections. Newman ('23) devotes two chapters (chap. IV and V) of his 'Physiology of Twinning' to the problem of monozygotic twins in fishes.

Symmetry reversal (*situs inversus viscerum*) in one member of a pair of conjoined fish embryos has been studied by Morrill ('19), by Swett ('21) and by Bovet ('31).

In addition to Stockard's methods for producing monstrosities in fishes by changes in the salt concentration, oxygen tension or temperature of the water surrounding the eggs, ultra-violet radiation has also been found effective in causing the development of conjoined twins (Henrichs and Genther, '31).

Some experimental work upon the physiological relations in double embryos has recently been carried out by Goetsch and Schindler ('34).

MATERIAL

The material upon which the present account is based consists of 3547 double embryos and 105 triple embryos of the brook trout, *Salvelinus fontinalis* and the rainbow trout, *Salmo irideus*. These appeared in the New Hampton hatchery of the New Hampshire Fish and Game Department in February, 1937. The author is greatly indebted to Mr. Earl E. Hoover for collecting these specimens and making them available for study.

OBSERVATIONS

Any system of classification of conjoined twins according to the degree of duplicity or the nature of the union must, of course, be of an arbitrary nature since in any large group of specimens, the individuals making up the various classes form a regular series passing from one type into the other. Some sort of systematic arrangement is necessary, however, for purposes of discussion and all of those who have dealt with any considerable number of specimens have proposed some scheme of classification emphasizing one or another feature of the type of union exhibited by these forms. The most familiar general terms which are used to designate the nature of the duplicity in cases of monoval twinning are: 1) anadidymus, doubling of the anterior end; 2) katadidymus, doubling of the posterior end; 3) anakatadidymus, doubling of both ends; and 4) mesodidymus, doubling in the middle portion of the body with the two ends single. These classes are somewhat too inclusive to be of use in analyzing the types of doubling encountered in fishes, the great majority of which fall into the single category of anadidymus or doubling of the anterior end, while yet exhibiting all degrees of variation as to the extent to which the anterior end is duplicated. Thus, Windle (1895) distinguishes nine different classes of anadidymi ranging from those in which the doubling extends only to the eye level, the adjacent eyes of the two heads being fused to form a single median eye, to those in which the two embryos are almost completely separated being united by their caudal extremities alone. A tenth group includes the examples of anakatadidymus, the cases of complete doubling, while in still another class are to be found those specimens in which one member of the paired individual is greatly reduced in size so as to appear as a 'parasite' of the other twin. Schmitt's ('01) classification emphasizes the nature of the attachment rather than the degree of duplicity, dividing the specimens according to whether the union is ventral, lateral, or of some intermediate type. Gemmill ('12) divides his specimens among nine classes based upon the anatomical

nature of the union, class I containing individuals in which the twin brains unite at the optic lobes; class II, those in which the two brains are united at the medulla, etc.

In the present study the specimens have been divided into five main groups according to the degree of doubling exhibited (table 1). All individuals in which the duplicity is confined

TABLE 1

			NUMBER OF SPECI- MENS	PER CENT OF TOTAL
A Union anterior to dorsal fin	1 Both com- ponents normal	I Union at eye level, 3 eyes	237	6.5
		II Union at eye level, 4 eyes	228	6.2
		III Union in pectoral region	210	5.7
		IV Union posterior to pectoral region	403	11.0
	2 One component defective or 'parasitic'		284	7.8
B Union posterior to dorsal fin	1 Both components normal		1393	38.1
	2 One component defective or 'parasitic'		488	13.4
C Union by yolk sac only	1 Both components normal		182	5.0
	2 One component defective		102	2.8
	3 Both components defective		14	0.4
D Doubling at posterior end (Katadidymus)			3	0.1
E Doubling at ante- rior and posterior ends with fusion in middle region			3	0.1
F Triple individuals	1 All three components normal		26	0.7
	2 Two components normal		35	1.0
	3 One component normal		35	1.0
	4 All three defective		9	0.2
Total			3652	100.0

to the region anterior to the dorsal fin are classed in group A, while those in which the doubling extends further posteriorly, to behind the dorsal fin, make up group B. Group A and group B are thus simply two different degrees of anadidymus, but 88.7% of all the specimens fall into these two classifications. Group C contains the cases of anakatadidymus; specimens in which there are two quite complete twins joined by

the single yolk sac. These, it will be noted, account for 8.2% of the total number of specimens. Thus only one case of anakatadidymus occurs for every eleven cases of anadidymus. Only three cases of posterior doubling, katadidymus, were found. These are included in group D. The individuals of the fifth and final group of twin specimens, group E, show a condition in which both anterior and posterior portions of the body are doubled while the middle region is single. These are in all probability cases of partial secondary fusion of two embryos originally quite separate.

It was thought advisable to further subdivide group A into four classes as follows: Class I includes the individuals which exhibit the least degree of doubling, the head being doubled only back to the eye level. The point of junction being at this level, the two adjacent eyes of the two heads are represented by a single median eye. Class II includes specimens in which the doubling extends somewhat further back so that four eyes are present instead of three, but the point of union is still in the head region anterior to the pectoral fins. The twin individuals of class III are united behind the head in the pectoral region so that there is duplicity of all head structures but only two pectoral fins are present, the adjacent fins being lacking. Class IV contains the specimens in which the point of union lies between the pectoral region and the dorsal fin. These embryos have four pectoral fins but only a single dorsal fin.

The final group in table 1, group F, embraces all the cases of triplicity. These, it will be noted, make up 2.9% of the total. In the present set of specimens therefore, monoval triplets occur in a proportion of one case to every thirty-five cases of monoval twins.

It has long been recognized in the study of teratology, that in cases of duplicity it is not uncommon to find that, while one member of the twin pair is a normally developed individual, the other member may show defects of one sort or another varying from relatively slight deformities to very abnormal conditions in which the weaker member of the pair appears as a mere small appendage or 'parasite' of its twin.

Such 'autosite-parasite' types are found in all but D and E of the main groups mentioned above but, as will be noted, occur with different degrees of frequency in these groups. They are listed in the table as a separate division under each group. It should be remarked that in a number of cases the two members of the pair are of nearly equal size but one shows some clearly marked defect such as the absence of one or both of the eyes. These are included in the 'autosite-parasite' groups in the tabulation.

TABLE 2

	NUMBER	PER CENT OF TOTAL		NUMBER	PER CENT OF TYPE
A Union anterior to dorsal fin	1362	38.4	1 Both normal	1078	79.1
			2 One defective	284	20.9
B Union posterior to dorsal fin	1881	53.0	1 Both normal	1393	74.1
			2 One defective	488	25.9
C Union by yolk sac only	298	8.4	1 Both normal	182	61.1
			2 One or both defective	116	38.9
D Doubling at posterior end	3	0.1			
E Doubling at both ends—middle fused	3	0.1			
Total	3547	100.0			

Some further analysis of the proportional relations between the various types of twinning is given in table 2. The cases of triple individuals are omitted from consideration here and the percentages given in the third column are calculated on the basis of the total number of twin forms, 3547. It will be noted that doubling of the entire anterior half of the body with the point of union lying behind the dorsal fin occurs in 53.0% of the cases. Duplicity of portions of the body lying anterior to the dorsal fin only, is found in 38.4%. Complete duplication, as previously pointed out, is relatively rare, occurring in only 8.4% of the twin specimens. When one examines

these groups with respect to the relative frequency of pairs in which one member is dominant while the other is parasitic or defective in some degree, one encounters the somewhat surprising fact that the tendency toward dominance of one member of a pair of monoal twins seems to be greater the greater the degree of separation between the individuals. Thus in class A where the twinning is found only in the anterior portion of the body, pairs showing defective members appear in the proportion of one to every four normal pairs. In class B, with more extensive twinning, the duplicity reaching to behind the dorsal fin, the 'autosite-parasite' condition is more common, one defective pair being found for every three normal pairs. Finally in class C, in which the two twins have entirely separate bodies, the number of defective pairs is greatest, the proportion being nearly two to three. These relations would seem to be of some importance in connection with certain theoretical considerations to be discussed in the next section. It should be stated that difficulty in distinguishing 'autosite-parasite' specimens in which the 'parasite' is nearly completely absorbed into the body of the normal twin might conceivably invalidate these figures. However the analysis has been made very carefully and the author believes that the striking results could not be accounted for in this way.

DISCUSSION

Consideration of any teratological specimen leads inevitably and at once to the question of its embryological origin and any discussion of such anomalies as those with which we are now concerned is fruitless if we lose sight of this question. In the words of Dareste (1891) 'La tératologie présuppose l'embryogenese.' On the other hand, if it is true that teratological specimens can only be correctly interpreted in the light of embryology, it is equally true that careful investigation of these anomalies often contributes much to our understanding of normal development. The mode of origin of these forms is of great interest to the embryologist since any general theory of embryonic development must account not

only for normal development but must also furnish a satisfactory explanation for the occurrence of these reduplications. Thus the study of twins and double individuals has played a significant part in the history of embryological theory from the earliest times. Aristotle recognized the importance of conjoined twins and pointed out that there are two possibilities as to their origin. They may come into being either by fusion of two originally separate embryos or by a process of fission of a single germ. These two alternative theories as to the origin of double monsters have formed the basis for a great amount of discussion even up to the present time. In the extended controversy between the adherents of the opposing theories of preformation and epigenesis the phenomenon of partial twinning was advanced by both sides as contributory evidence for the correctness of their views. The preformationists maintained that these anomalies arose by secondary partial fusion of two originally separate preformed embryos while the supporters of the theory of epigenesis largely held to the idea of fission, though admitting that partial fusion would account for some of the cases observed (Meckel, 1815).

Later, with the rise of the concrescence theory as enunciated by His (1876), interest in conjoined twins was again aroused and extended explanations of the method of formation of double individuals in the light of the concrescence theory were offered by Rauber (1877, 1878, 1879, 1880), and others. According to this theory the future head of the embryo is represented by the embryonic shield while the lateral elements of the trunk and tail are formed by the coming together of the two sides of the germ ring. Thus the future right and left sides of the body region are at first widely separated and only gradually approach each other, to fuse from before backward as development proceeds. This gradual fusion of the two halves is the process of concrescence. If this theory were true and if one should assume that for some reason two embryonic shields instead of one should arise on one egg it is obvious that a double or conjoined twin would result. The two separate embryonic shields would form two separate

head ends; as concrescence proceeded the two heads would be in competition, so to speak, for the portion of the germ ring lying between them. It may be assumed that each might gain half of this intermediate region, but that once the whole region is used up then the two embryos being formed must perforce be united at that point, and the remainder of the body will be single, formed by the lateral parts of the germ ring. The point of union would thus depend upon the original degree of separation between the two embryonic shields. Only if these were at opposite sides of the germ ring, separated by 180° , could the twins be entirely separate, united only by their attachment to the same yolk sac. Although Rauber strongly urged this explanation of the formation of double individuals as convincing evidence for the correctness of the concrescence theory Schmitt ('02), as a result of a study of serial sections of a number of double embryos at various stages of development, maintained the opposite view. He considers that the evidence indicates that these individuals are always entirely separate when first formed and that the conjoined condition is established by partial fusion and later disappearance of parts already formed. He thus maintains that the cases of conjoined twins constitute in reality an important piece of evidence against the theory of concrescence.

The ultimate abandonment of the concrescence theory in its original form came as a result of the experiments of Kopsch (1899, '04), Morgan (1893, 1895), and Sumner ('04) but the question of fusion or fission as the method of formation of conjoined twins was still persistent.

Gemmill ('12) in reviewing his own extensive work on this subject as well as that of others decides for the idea of fusion. He says (p. 6): "The recorded observations indicate that double monster fishes always arise on a single yolk, and from a single blastoderm at the margin of which two more or less separate centres of gastrulation and embryo-formation have made their appearance." If the twin centers of embryo-formation are sufficiently close together then, "The twin adjacent axes are inevitably brought together

posteriorly through the disappearance of the interval between them. . . . When the union is a purely lateral or an approximately lateral one, the posterior united part finally becomes simply and perfectly bilateral. This takes place through the gradual fusion and disappearance of structures belonging to the left and right halves respectively of the right and left component embryos."

Meanwhile the experiments of Driesch (1892), Morgan (1895) and others on the separation of blastomeres of the eggs of both vertebrates and non-vertebrates clearly demonstrated the possibility of producing twins and double individuals by this method and for a short time this was considered the most plausible general explanation of the occurrence of monozygotic twins in nature (Wilder, '04). It was soon demonstrated, however, that such individuals frequently do not arise until stages much later than those of cleavage and it has since been shown experimentally that double monsters can also be produced by constriction of eggs in the gastrula stage (Spemann, '03).

Stockard ('21) in an extensive monograph discusses the results of the work which he has carried out over a period of years on the effects of changing the environmental conditions on the developing eggs of *Fundulus* and advances a theory of twinning based on these results. Among the various types of monsters produced in these experiments were a number of cases of twins and double embryos. Since such individuals are extremely rare in nature in this species it may be assumed that those found among the experimental groups were all produced as a direct result of the experimental procedure. A number of methods, all tending to lower the developmental rate, were applied to these eggs and it was found that the double individuals appeared only in those groups of eggs which were treated during cleavage or before the formation of the germ ring. Specimens treated after the time of gastrulation never exhibited this type of anomaly. Stockard is led to believe that the stage of development is the one determining factor for the type of monster produced and not the kind of

treatment. He considers that these twins and double embryos arise as a result of the brief developmental arrest to which the eggs have been subjected during a specific critical period. When the eggs are allowed to resume their development the normal unequal rates of growth of the various parts are disturbed and as a consequence of this disturbance of the developmental balance, a process of budding occurs. This budding process is analogous to that which is seen in plants when the growing tip is destroyed; in such a case other potential buds which have previously been inhibited will express their capacity to grow. Thus the germinal disc is regarded as a stock along the periphery of which are a number of potential points any one or all of which might give rise to a budding embryo. Ordinarily, one of these points possesses an advantage over the others and gives rise to a growing embryonic process. The growth of this embryonic bud then dominates the entire germinal area and completely suppresses other potential points of growth. If however, the advantage of the primary growth point is weakened by suitable treatment at the proper time, it fails to dominate completely and a second point may bud into a growing axis. Twins will then result. Polyembryony could thus be regarded as a type of asexual reproduction and Stockard points out that since all eggs presumably have the potentiality for thus giving rise to two or more individuals this really establishes the phenomenon of 'alternation of generations' as universal among animals.

Newman ('23) objects to Stockard's theory of budding and favors rather the idea that conjoined twins arise by fission, the two components of the double embryo being derived from the bilateral primordia of a single individual. Twinning is thus "essentially a phenomenon involving a physiological isolation of equivalent parts of the blastoderm and a regulation of the isolated or twinned regions into complete embryos." As to the cause of this physiological isolation he is in essential agreement with Stockard that temporary cessation or radical retardation of development at a critical period is the most

important feature. Newman considers that the rather frequent occurrence of situs inversus viscerum in one member of a pair of conjoined twins speaks strongly for the view that the two members come originally from two primordia that normally give rise to structures which are mirror-images of each other. He maintains that twinning is a form of axiate reproduction and cannot be properly considered as an asexual phase in an 'alternation of generations.'

Henrichs and Genther ('31) found that ultraviolet radiation of the eggs of *Fundulus heteroclitus* just before the first cleavage brings about the production of numerous twin individuals. They believe that their experiments point to the origin of duplicities "by fission of an original axis (more or less complete) followed by secondary lateral fusions which may involve some melting together of internal structures."

With these theories of the origin of double individuals in mind we may turn now to some of the more important relations observed in the present series of specimens.

The fact that anadidymus occurs in by far the greater number of the animals discussed in this paper has already been noted. This agrees with all earlier reports. Cases of separate twins were found much less frequently; in 8.2% of the specimens. Windle (1895) found only two cases of anakatadidymus in forty-six specimens of doubled forms, while Gemmill ('12) reports seven out of seventy-one. Stockard ('21) found complete separation occurring in a proportion of one to every eight specimens of conjoined forms. It thus appears that Stockard's conclusion that the formation of twin embryonic shields on opposite sides of the egg occurs in about 10% of the cases of twinning is approximately correct.

A more interesting feature of the collection under discussion is the matter of the distribution among the various classifications of pairs having one defective member. It has already been pointed out that there is a definite indication that 'autosite-parasite' pairs are more frequent the greater the degree of separation between the two members of the pair. This is in opposition to Stockard's ('21) statement that "The degree

of doubleness bears no relation to the perfection of development of the two components. 'Two heads, although slightly separated on a single body, are both just as frequently normal in structure as are the two components of almost completely double specimens.' In the light of the figures given in table 2 it would seem that such slightly separated twins are much more frequently normal than are the components of almost or quite completely double specimens. This has in fact, been briefly remarked by Newman ('23, p. 53) in the statement that "when the duplicity extends farther toward the posterior end—there is a greater tendency for one component to be abnormal," but he attempts no explanation of the fact. The establishment of the validity of this conclusion is of much importance since it has a direct bearing upon the theories of the mode of origin of monoal twins in general. As Stockard has pointed out, the two components of these conjoined twins are definitely known to be derived from a single fertilized egg and to be, therefore, of identical genetic constitution. Since also, the environments of the two components cannot be significantly different, it is clear that the defects exhibited by one twin are of ontogenetic origin and must be attributed to something in its relations to the other twin or in its mode of origin. Stockard maintains that the cause must be sought in a developmental competition between the two members of the pair. The larger component, having an initial advantage, exerts an inhibiting influence upon the other, the inhibition being mainly the result of a slower developmental rate in the defective twin. This accords with his theory of 'budding' and an analogy is drawn between this process and the inhibition exerted by the apical bud of the growing plant stem over the axillary buds. On the basis of this idea there seems no logical way of accounting for a greater frequency of defective pairs in the more widely separated twins unless perhaps we could assume that the greater degree of separation implied earlier time of separation. If such were the case it might be conceivable that embryos which come into competition earliest would have the greater opportunity for exerting an inhibiting effect at the

most critical stages and would thus produce the greatest proportion of defective pairs. This idea seems untenable, however, for Morrill ('19) has shown that the different degrees of doubleness are not at all connected with different times of origin of the twinned condition.

Newman's ('23) explanation of unequal development of the two components of a pair rests upon his fission theory of origin. Since he maintains that conjoined twins always arise by partial separation of the two bilateral halves of a single embryonic shield as a result of lowered metabolic rate at the time when the axis is established, he considers it logical to assume that after physiological isolation, the primordium of one-half of the axis may be more severely inhibited and hence develop less normally than the other. He cites the frequent occurrence of unilateral abnormalities in experimentally produced monsters as evidence that such differential inhibitions are possible. Once one twin is thus relatively inhibited, secondary influences from the stronger twin passed through the medium of the common circulation may further suppress its development. The fact that situs inversus viscerum and mirror imaging are rather commonly found in one member of the pair in conjoined twins furnishes a strong support for this theory.

It would seem that there is at least a possibility of accounting for the appearance of defective pairs in greater numbers in the more widely separated twins on the basis of Newman's theory. If the abnormality of one component rests upon an initial difference between the primordia which formed the twins rather than upon an inhibiting action by the stronger member of the pair, it might not be surprising if the number of pairs showing defects would vary directly with the extent of separation of these two diverse primordia. Unfortunately, no detailed study has yet been made of the occurrence of situs inversus in the present series. When this is done it may serve to throw additional light on the matter.

One further subject of special interest in the specimens under consideration remains to be discussed. This concerns

the cases of monoval triplets. Examples of this type are infrequently met with and only a few records are to be found in the literature. Lereboullet (1863) reported a specimen of *Esox lucius* with three separate heads and several cases of triplicity in trout were described by Klaussner (1890), Schmitt ('01) and Rauber (1880). Gemmill ('12) found only one example of triplets as compared with seventy double forms and Stockard ('29) records that he has seen only two triple individuals in his examination of thousands of eggs. In view of this paucity of specimens in previous collections, it is somewhat surprising to find cases of triplicity making up 2.9% of the individuals in the series now under discussion. These triple embryos show all the various degrees of separation that are to be found among double individuals. A brief classification of the types is given in table 3. All the cases are included here except that of one individual in which katadidymus was found, there being two separate embryos one having a double tail. Aside from this single example all the specimens of triple embryos exhibit either anadidymus or anakatadidymus. The percentage of cases in which all three embryos are complete, joined by the yolk sac only, agrees rather closely with the percentage of anakatadidymi among double embryos. Cases in which two embryos are conjoined while one is separate make up about one-fourth of the total number of triplets while most (over two-thirds) of the specimens are examples in which the three separate anterior ends are joined to a single tail. These latter are perhaps the only cases to which the term 'conjoined triplets' may be properly applied. Usually, in such specimens, the three components do not diverge from the same point but show two different points of union. In more than half the cases (forty-two) the two points of union lie, one anterior and one posterior to the dorsal fin, but it will be noted that it is not uncommon to find all three heads united anterior to the dorsal fin level or all three united posterior to it.

The origin of triple embryos is, of course, subject to the same theoretical interpretation as is that of double embryos. Stockard regards it as simply a further degree in multiple budding

and explains the rarity of these cases by assuming that "one point of gastrulation, or embryo formation, has an extremely high tendency to prevent or suppress the existence of any other such point of excessive proliferation. When a second point is capable of expression the two almost without fail completely

TABLE 3

TYPE	NUMBER	DEGREE OF SEPARATION	NUMBER	NUMBER OF COMPONENTS NORMAL	NUMBER		
a 3 separate embryos	6 = 5.8%		6	3 2 1 0	1 0 3 2		
b 2 embryos united; 1 separate	28= 26.9%	2 united anterior d.f.; 1 separate	21	3 2 1 0	4 8 7 2		
		2 united posterior d.f.; 1 separate	7	3 2 1 0	1 1 3 2		
		c 3 embryos united	70 = 67.3%	3 united anterior to dorsal fin	17	3 2 1 0	5 8 4 0
				2 united anterior to d.f.; 1 poste- rior	42	3 2 1 0	14 15 12 1
3 united posterior to d.f.	11			3 2 1 0	1 3 5 2		
Total				104			

dominate the growth capacity of the entire germinal region and triplets are the rarest exception." Newman ('23), though he does not discuss cases of triplicity in detail, remarks (p. 2) that "Rarely does a division into more than two individuals occur, unless one of the two redivides."

As in the case of double embryos, there seems to be a rather definite indication in the present series of specimens that 'parasitic' or defective embryos occur most commonly in the individuals in which the degree of separation of the three components is greatest. This may be seen by examining the figures in the last row of table 3. In every classification in which the point of attachment of individuals is anterior to the dorsal fin, most of the embryos fall into the groups in which either two or all three of the components are normal. On the other hand, in those classifications in which union is posterior or in which all three embryos are separate, most individuals show defects in either two or all three components. A more

TABLE 4

	TRIPLE EMBRYOS				DOUBLE EMBRYOS			
	Total number	Number normal	Number defective	Percent-age defective	Total number	Number normal	Number defective	Percent-age defective
Separate embryos	46	22	24	52.2	596	517	79	13.3
Embryos united posterior to d.f.	131	73	58	44.3	3762	3518	244	6.5
Embryos united anterior to d.f.	177	129	48	27.1	2724	2582	142	5.2

exact analysis may be obtained if one considers each individual case separately. This has been done in table 4 for both double and triple forms. The first row of figures in this table gives the total number of component embryos belonging to each type among the triple forms. Thus there are forty-six embryos which are complete; united with others only by attachment to a common yolk sac. Of these, twenty-eight are found on the same egg with a conjoined pair (group b of table 3) while eighteen are found on the six eggs of group A. Examination of the specimens shows that of the forty-six separate embryos only twenty-two are normal while twenty-four show defects ranging from absence of one eye to great abnormality and reduction in size. The other figures in the table are arrived

at in the same manner and comparison of the percentages of defective embryos among the triple embryos reveals the general tendency already alluded to, toward abnormality in the more widely separated components. However, by referring to the similar percentages for double individuals given in the same table one sees that all types show much higher percentages of abnormality in the cases of triplicity than in the cases of duplicity. Apparently 'competition' with two other embryos is more difficult and less likely to be completely successful than is competition with only one other embryo on the same egg.

SUMMARY

1. 3547 double embryos and 105 triple embryos of the brook trout, *Salvelinus fontinalis* and rainbow trout, *Salmo irideus* from the New Hampton hatchery of the New Hampshire Fish and Game Department have been analyzed as to the relative numbers of individuals showing various types of twinning and triplicity.

2. Anterior doubling, anadidymus, is by far the commonest type of duplicity, being found in 91.4% of the twins; 8.4% show complete separation of the twin bodies, anakatadidymus.

3. Conjoined twins which are united anterior to the dorsal fin show fewer defective pairs than do those which are united posterior to the dorsal fin level and the latter show fewer defective pairs than do completely separated twins.

4. Triple embryos show all the various degrees of separation of the components that are exhibited by double individuals and there seems a definite indication that here too the percentage of defective embryos is greater the greater the degree of separation of the components.

5. The percentage of defective embryos in all groups of triple embryos is much greater than that in similar groups of double forms. Thus an embryo developing on the same egg with two others has much less chance of being normal than does an embryo in competition with only one other.

LITERATURE CITED

- ALDROVANDI, U. 1642 *Monstrorum historia*. 429 pp. Bologna.
- BOVET, D. 1931 L'orientation des viscères chez les truites doubles. *Bull. Biol. France et Belgique*, T. 65, pp. 216-233.
- DARESTE, C. 1891 *Recherches sur la production artificielle des monstruosités*. 469 pp. Paris.
- DEAN, B. 1916 *A bibliography of fishes*. 3 vols. New York.
- DRIESCH, H. 1892 *Entwicklungsmechanische Studien III-VI*. *Zeits. f. Wiss. Zool.*, Bd. 55, S. 1-62.
- GEMMILL, J. F. 1912 *The Teratology of Fishes*. 73 pp. Glasgow.
- GOETSCH, W., AND O. SCHINDLER 1934 a *Doppelbildungen bei Fischen*. *Sitzungsber. der Ges. f. Morph. u. Phys. München*, Bd. 43, S. 35-42.
- 1934 b *Entwicklungsphysiologische Untersuchungen an Fischlarven I Mitteilung. Beobachtungen und Versuche an Forellenzwillingen*. *Arch. f. Entw.*, Bd. 131, S. 483-511.
- HENRICHS, M. A., AND I. T. GENTHER 1931 Ultra-violet radiation and the production of twins and double monsters. *Physiol. Zool.*, vol. 4, pp. 461-485.
- HIS, W. 1876 *Untersuchung über die Entwicklung von Knochenfischen, besonders über diejenige des Salms*. *Zeits. f. Anat. u. Entwges.*, Bd. 1, S. 1-40.
- KLAUSSNER, F. 1890 *Mehrfachbildungen bei Wirbelthieren*. 71 pp. München.
- KOPSCH, F. 1899 *Die Organization der Hemididymi und Anadidymi der Knochenfische und ihre Bedeutung für die Theorien über Bildung und Wachstum der Knochenfischembryos*. *Internat. Monatschr. Anat. u. Phys.*, Bd. 16, S. 221-263.
- 1904 *Gastrulation und Embryobildung bei den Chordaten*. I. Leipzig.
- LEREBOULET, A. 1863 *Sur les monstruosités du brochet*. *Ann. Sci. Nat. (Zool.) Paris*, Ser. 4, T. 20, pp. 177-271.
- MECKEL, J. F. 1815 *De duplicata monstrosa commentarius*. Halle u. Berlin.
- MORGAN, T. H. 1893 *Experimental studies on teleost eggs*. *Anat. Anz.*, Bd. 8, S. 803-814.
- 1895 *The formation of the fish embryo*. *J. Morph.*, vol. 10, pp. 419-472.
- 1895 *Half-embryos and whole-embryos from one of the first two blastomeres of the frog's egg*. *Anat. Anz.*, Bd. 10, S. 623-628.
- MORRILL, C. V. 1919 *Symmetry reversal and mirror imaging in monstrous trout and a comparison with similar conditions in human double monsters*. *Anat. Rec.*, vol. 16, pp. 265-291.
- NEWMAN, H. H. 1923 *The Physiology of Twinning*. 230 pp. Chicago.
- RAUBER, A. 1877 *Die Theorien der excessiven Monstra*. *Virchow's Archiv*, Bd. 71, S. 133-206.
- 1878 *Die Theorien der excessiven Monstra*. *Zweiter Beitrag*. *Virchow's Archiv*, Bd. 73, S. 551-594.
- 1879 *Formbildung und Formstörung in der Entwicklung von Wirbelthieren*. *Morph. Jahrb.*, Bd. 5, S. 661-705.
- 1880 *Formbildung und Formstörung in der Entwicklung von Wirbelthieren*. *Morph. Jahrb.*, Bd. 6, S. 129-184.

- SCHMITT, F. 1901 Systematische Darstellung der Doppelembryonen der Salmoniden. *Arch. f. Entwm.*, Bd. 13, S. 34-134.
- 1902 Über die Gastrulation der Doppelbildungen der Forelle, mit besonderer Berücksichtigung der Concresezenztheorie. *Verh. d. Deutschen Zool. Ges.*, Bd. 12, S. 64-83.
- SPEMANN, H. 1903 Entwicklungsphysiologische Studien am Triton—Ei. III. *Arch. f. Entwm.*, Bd. 16, S. 551-631.
- STOCKARD, C. R. 1921 Developmental rate and structural expression; an experimental study of twins, double monsters and single deformities, and the interaction among embryonic organs during their origin and development. *Am. J. Anat.*, vol. 28, pp. 115-277.
- 1921-1922 The significance of modifications in body structure. *The Harvey Lecture Series*, XVII, pp. 23-64.
- 1929 The problem of development in adult constitution. *Cornell Univ. Med. Coll. Studies*, vol. 14, pp. 1-27.
- SUMNER, F. B. 1904 A study of early fish development, experimental and morphological. *Arch. f. Entwm.*, Bd. 17, S. 92-149.
- SWETT, F. H. 1921 Situs inversus viscerum in double trout. *Anat. Rec.*, vol. 22, pp. 183-199.
- WILDER, H. H. 1904 Duplicate twins and double monsters. *Am. J. Anat.*, vol. 3, pp. 387-472.
- WINDLE, B. C. A. 1895 On double malformations amongst fishes. *Proc. Zool. Soc. London*, pp. 423-429.

PLATE 1

EXPLANATION OF FIGURES

- A A series of photographs showing various types of double embryos. Natural size.
- B A series of photographs showing various types of triple embryos. Natural size.



A



B

THE CYTOLOGICAL RELATIONSHIP BETWEEN THE HYPOPHYSIS AND THE GERMINAL EPITHELIUM OF THE TESTIS ¹

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FIVE TEXT FIGURES AND TWO PLATES (TWELVE FIGURES)

INTRODUCTION

The relationship between the anterior lobe of the hypophysis and the gonads has been investigated from the anatomical, histological, physiological and more recently the cytological aspects.

Gatz ('33 and '36), Severinghaus ('33), and Guyer and Claus ('34) were among the first workers to report on the effects of gonadectomy on the cytological structure of the basophilic cells of the anterior hypophysis in male rats. They found that the basophiles are much more numerous and present a distinct modification in their distribution. The cells have no apparent arrangement in the hypophysis of the normal rat but following gonadectomy, they frequently become arranged in cords which may or may not take a position adjacent to a blood capillary. The Golgi material, which is located near the margin of the eccentric nucleus, the cytoplasm, and the nucleus have increased in size. Vacuoles of varying sizes appear in the basophiles 2 months after gonadectomy. The Golgi material, if in the shape of a 'body,' is always found between the nucleus and the vacuole.

¹ This paper, together with another (Anat. Rec., 1937, vol. 69, p. 429), was submitted to the graduate school of the University of Minnesota in partial fulfillment of the requirements for the degree of doctor of philosophy, in July, 1936.

Gatz noticed that the basophiles could be arbitrarily separated into three groups of cells; those appearing normal, those which had increased in size tremendously, and those which contained large vacuoles of granular substance. The Golgi material is not a static substance. In many of the cells, small girdles of osmiophilic substance surrounding a secretion droplet can be observed in the Golgi material. In the vacuolated cells, the Golgi substance presents a varied structure. It is either in the form of a discrete 'body' located between the vacuole and the nucleus, or in the form of small osmiophilic girdles and particles surrounding the vacuole, or frequently is a combination of both types. The secretion within the vacuoles is thought to arise from the Golgi material.

Severinghaus ('33) thought that a relation should exist between the Golgi material and the heightened secretory activity of the basophiles but said,

Neither the Golgi material nor the mitochondria can be related with certainty to the elaboration or discharge of specific secretory products in the cells of the anterior pituitary of the rat. From my own observations it appears that this "ringbildung," which is the Golgi apparatus, takes no part in the formation of the vacuole in the castration cell, but is itself pushed aside with the nucleus as the vacuoles enlarge and coalesce. . . . it does not play a direct part in the formation of the vacuole of the castration cell.

Guyer and Claus ('34) were convinced,

that in the particular material with which we have worked, the vacuolar substance arises as a secretion within the Golgi framework or through its utilization as a center of transformation. At the onset of the enlargement in such basophile cells, a series of transparent, highly refractive globules, or sometimes somewhat irregular shaped bodies, appear within the strands of the Golgi meshwork, particularly at points of intersection. Often series of these hyaline bodies are also visible within the individual strands, giving the appearance of a row of beads. Eventually clear looking globules seem to transude from the meshwork and pass outward into the surrounding cytoplasm.

Guyer and Claus ('32 and '37) studied the effects of the implantation of cancerous tissue and of thyroidectomy on the anterior pituitary. The vacuolated cells, which are found following the implantation of cancerous tissue and gonadectomy are secretory in nature while the vacuolated basophiles occurring after thyroidectomy are caused by cellular degeneration. The Golgi material is not located in its normal position in the latter group, very frequently being widely separated from the nucleus.

MATERIAL AND METHODS

The tissues used in this study were the hypophyses and testes of male albino rats. The animals were reared in the laboratory from purchased stock and from animals of the old colony. Litter-mate animals were used for controls in all of the experiments. The food consisted of a mixture of chicken mash and mink food which was supplemented by the addition of cod liver oil, milk, alfalfa and vegetables. The vitamin content of the diet was consequently maintained at a satisfactory level, which was attested by the appearance of normal germinal epithelium and the successful growth of all litters reared in the laboratory.

The entire testis and epididymis were removed from the rat in the gonadectomized group. In the artificial cryptorchid animals, the testis and the epididymis were forced through the inguinal canal into the body cavity. The inguinal canal was then ligatured to prevent the re-descent of the testis into the scrotum. Infrequently, the entire inguinal canal was not secured in the ligature and some of the tissue underwent hypertrophy allowing the testes to return into the scrotum. The animals, in which this occurred, were readily detected and were not used in the collection of data.

The procedure used for gonadectomy was employed in the experiments on vasectomy and ligature of the vas deferens. The vas deferens was freed of the accompanying blood vessels and nerves and was ligatured by means of a silk thread so that the occlusion of the duct remained permanent. In vasectomy, a small section of the vas deferens, $\frac{1}{8}$ to $\frac{1}{4}$ inch in length,

was removed. The testes remained in their normal position in the scrotum.

In the experiments on irradiation, the testes and the scrotum were the only parts of the body exposed to the x-rays. The irradiation was done under the supervision of Dr. K. Wilhelm Stenstrom, associate professor of biophysics at the University Hospital. The animals were given a dosage of 1200 R. units. The irradiation was administered at a voltage of 100 kilovolts and at an amperage of 8 m.a. The target distance was 30 cm. A 1-mm. aluminum filter was used. The animals were securely fastened in a leaded box with a single opening, at which point the scrotum was exposed. The operation required a period of $17\frac{1}{2}$ minutes. In another series of animals the same specifications, which have been recorded, were used with the exception that the target distance was decreased to 20 cm. The time of dosage was decreased to $9\frac{3}{4}$ minutes.

The colony contained some old males which were sacrificed to determine the effect of age on the pituitary and testes. Unfortunately no records of their exact age were available. However, the degenerate condition of the germinal epithelium was not caused by a vitamin deficiency since these animals received the same diet as the other members of the colony. The onset of their sterility was observed when they failed to mate with females.

The testes were fixed in Bouin's fluid to which a few crystals of urea had been added. They were dehydrated in alcohol, cleared in xylol, and embedded in paraffin. Serial sections were cut at 7μ and stained with Heidenhain's iron hematoxylin.

Some of the pituitaries were fixed by the osmium tetroxide method of Mann Kopsch (Weigl), modified by Ludford, to demonstrate the positive image of the Golgi material. The details of the technique may be found in Guyer's 'Animal Micrology.' It was necessary to fix the tissue immediately (1 to 2 minutes) after the death of the animal or the resultant impregnation with osmic acid was imperfect. The hypophyses were kept at a temperature between 33° and 34° C. during the

3 days in which they were in osmic acid, under darkened and lighted conditions. The light factor seemingly had no effect upon the subsequent impregnation. The tissue was embedded in paraffin and serially sectioned at a thickness of 5μ .

The sections which were prepared by the above method exhibit a cytoplasm which has a yellow-tan appearance. The nuclear membrane, which is stained brown, and the chromatin granules, which are tinged a light brown, outline the nucleus very clearly. The periphery of the Golgi material is blackened and the lighter central portion of the 'body' contains numerous osmiophilic granules, girdles and threads. It was frequently necessary to bleach the sections with a weak solution of hydrogen peroxide (2 drops of peroxide to 50 cc. of water) so that the minute details in the periphery of the Golgi material could be studied.

The remainder of the pituitaries were fixed following the method of da Fano, without the subsequent impregnation with silver nitrate, to disclose the so-called 'negative image' of the Golgi material or the 'ringbildung.' The above method may be found in Lee's 'Microtometist's Vade-Mecum.' The hypophyses were fixed in cobalt nitrate and neutral formalin. The tissue was washed in distilled water, dehydrated in alcohol, cleared in xylol, and embedded in paraffin. Serial sections of 5μ were cut and stained with aniline blue, Delafield's hematoxylin, acid fuchsin, eosin and orange G.

I wish to gratefully acknowledge my indebtedness to the director of this investigation, Dr. J. E. Wodsdalek, for his interest and suggestions during the progress of the work and the writing of the report and to the zoology departments of the University of Minnesota and Carleton College for the animals and materials which were supplied. I am also indebted to Dr. K. W. Stenstrom under whose direction the rats were irradiated.

RESULTS

Most workers agree that there are three types of cells present in the anterior lobe of the hypophysis; the chromophobes or stem cells, the acidophiles, and the basophiles. These cells may be differentiated from one another by the size, shape, density and position of the Golgi material (Atwell, '29 and '32; Severinghaus, '33), as well as by their specific granules. The chromophobe cells are thought to give rise to the two types of chromophilic cells, Severinghaus ('33) and others. In the present study, the Golgi material of the chromophobe was either an elongated network, closely applied to the nucleus, or a spherical mass which was free in the cytoplasm. As a consequence it was impossible to make a study of their comparative sizes in the various groups of animals. The present paper, therefore, treats with the detailed study of the characteristics and the size of the Golgi material in the basophiles and the acidophiles of the pars anterior of the hypophysis of the male rat under the various conditions which have been enumerated.

In order to determine the magnitude of the changes occurring, the size of the cell, the nucleus, and the Golgi material was measured. The cells must be carefully selected for the measurement of the Golgi material because the worker must be certain that the structure is cut through its center. The practice of the writer has been to proceed in an orderly manner back and forth across a chosen section measuring each cell in which the Golgi material appeared to be cut through its center and at the same time the type of the cell could be determined.

Condition of the germinal epithelium. The average size of the normal testis is 10.5×21 mm. for the group of rats which were used in this study. The seminiferous tubules of the normal testis were lined by a stratified layer of germinal epithelium, which surrounded a lumen containing sperm cells. The tubules were compact in arrangement and presented small intertubular spaces in which a few interstitial cells were scattered (fig. 6).

The testes of x-radiated animals showed a progressive atrophy, decreasing in size to 8×14 mm. after 2 months. The shrunken, irregularly shaped tubules are lined with a vacuolated fibrillar type of cytoplasm with large pycnotic nuclei. There are no meiotic figures, sperm, or any cell differentiation. The intertubular space, which is increased in area is filled with either an accumulation of granular lymphoid material or interstitial cells, which appear to be more numerous (fig. 7). However the interstitial cells may only appear to have increased because of the atrophy of the seminiferous tubules since very few mitotic figures were observed in the cells.

The testes of the vasectomized animals decreased in size to 8×15 mm. after a period of 2 months. This group showed a varied result. Some of testes appear to be normal in size and contain sperm cells and mitotic figures, while other gonads appear atrophied and degenerate (fig. 8). Regardless of this confusing result, the pituitary response could readily be correlated with the condition of the testes.

The testes of the cryptorchid animals are 7×14 mm. in size after a period of 2 months. The tubules are shrunken and are lined with a vacuolated fibrillar cytoplasm. There are two rows of cells which have pycnotic nuclei and a deeply stained cytoplasm in a few of the tubules. No meiotic figures are present in any of the sections. The intertubular space is increased in size and has the same appearance as that of the x-radiated animals. Mitotic figures are not observed in the interstitial tissue, even though the cells appear to be more numerous (fig. 9).

The average size of the senile rat testes is 7.7×15 mm. Histological examination of the testes shows that the tubules are shrunken and that they contain no normal germinal epithelium. The gonads are very much like those of the artificial cryptorchid or the irradiated animal failing to exhibit meiotic figures or sperm (fig. 10). The enlarged intertubular space is filled with a lymphoid accumulation and cords of interstitial cells.

Cytological modifications of the acidophiles. The Golgi material of the acidophile of the normal animal has the appearance of a much coiled, compact net, which is twice as long as it is wide. The long surface of the material forms a cap on the nucleus. The density of the acidophilic Golgi material is much greater than that of the basophilic material. Reference to figure 1 shows that the acidophiles have decreased in size in all cases following the loss of the germinal epithelium. The decrease in size was not very pronounced except in the case of gonadectomy. The Golgi material retained its characteristic compact, dense, net-like appearance and also its posi-

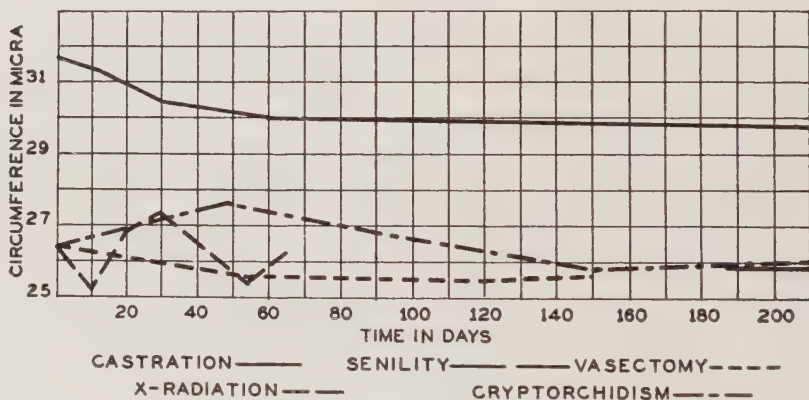


Fig. 1 Circumference of the acidophilic cells in the anterior lobe of the rat hypophysis.

tion adjacent to the nucleus. The Golgi substance does decrease slightly in size, which is shown in figure 2. As in the case of the cytosome, the decrease is most pronounced after gonadectomy.

Cytological modifications of the basophiles. The basophiles of the normal animal usually are more or less uniform in size and structure, although infrequently an enlarged vacuolated basophile may be seen. The Golgi material is oval to round in shape and is located very near the border of the eccentric nucleus. The material usually does not cap the nucleus as it does in the case of the acidophile. The Golgi substance has a

loose structure being composed of an osmicated peripheral portion, which has a thread-like appearance, enclosing a central mass or macula. The macula may contain some osmicated granules, threads, and girdles in the normal basophilic cell. However, the latter structures are more readily seen in the basophilic cells of the experimental animals.

The basophiles are more numerous in the rat hypophysis following the loss of the germinal epithelium, which has also been shown by Addison ('17) after castration; by Severinghaus ('33) after castration; by Guyer and Claus ('34) after castration; and by Martins and de Mello ('35) after castration

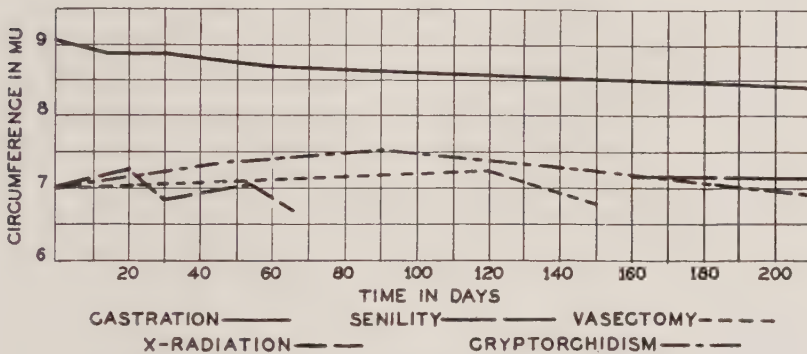


Fig. 2 Circumference of the Golgi material in the acidophiles in the anterior lobe of the rat hypophysis.

and artificial cryptorchidism. Following the loss of the germinal epithelium, the basophiles frequently are found distributed in cord-like masses (fig. 15).

In the experimental animals, the basophiles may be classified into three groups of cells and the individual cells of each group show the same characteristics and are of about the same size. The basophiles of the first group appear much as they do in the hypophysis of the normal animal. The cytoplasm appears more or less homogeneous, the nucleus is of the normal size, and the Golgi material which has not undergone hypertrophy is located in the normal position. In some cases the periphery of the Golgi material showed numerous

evaginations containing droplets. These cells are about the size of the basophiles which are found in the normal gland. The second group of basophiles are larger cells and present a cytosome which is filled with numerous small granules of osmiophilic substance. All the components of the cell increased in size to the same degree during the first 2 months following the loss of the germinal epithelium, after which the hypertrophy of the nucleus is not comparable to that of the cytosome.

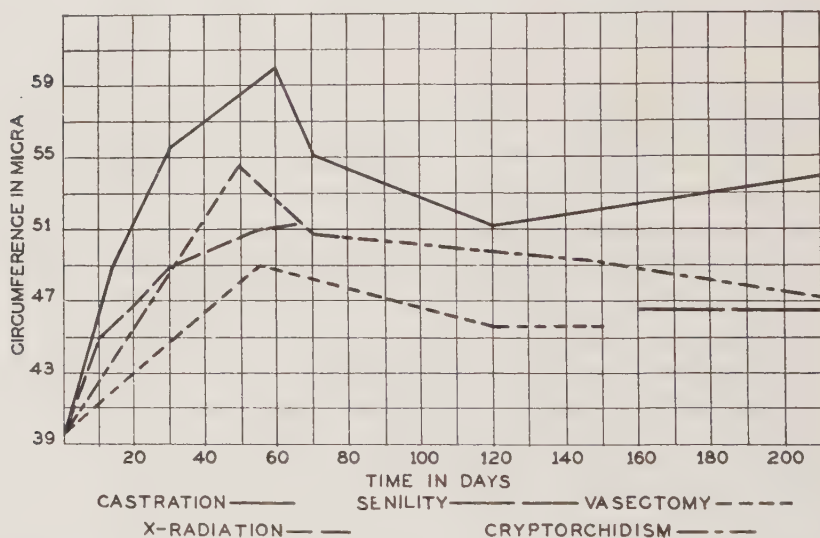


Fig. 3 Circumference of the basophilic cells in the anterior lobe of the rat hypophysis.

Reference to figure 3 shows that the average size of the basophiles is decreasing after the second month. The graph, however, fails to record the large basophiles observed and measured since the curve also includes the smallest cells. The size of the basophilic cells 15 months after gonadectomy shows a peak beyond that shown for the 2-month period. In the 15-month period, the measurements are almost entirely from the much enlarged basophilic cells, since very few of the basophiles are of normal size. The basophiles are much more

numerous after the 15-month period also. The graph likewise shows that gonadectomy brought about the greatest hypertrophy and that vasectomy causes the least. In all cases, except gonadectomy, there is interstitial tissue and some degenerate germinal epithelium present in the testes. These cells apparently provide some inhibitory factor and consequently the hypertrophy of the basophilic cells is not as great.

A modified type of cell becomes noticeable 2 months after gonadectomy—a large vacuolated basophile which has been called the ‘castration cell.’ The cells are much larger than the other types of basophiles seen in the anterior lobe. There are numerous intermediate stages between the basophile and the vacuolated cell. Many of the basophiles show numerous small vacuoles which coalesce to form the large vacuole of the vacuolated cells. The vacuolated cells are signet-ring shaped in appearance. The cytoplasm forms a narrow rim about the edge of the vacuole and contains the nucleus and the Golgi material. The latter substance, when present as a distinct ‘body,’ is always located between the nucleus and the vacuole. The vacuoles usually are filled with a finely granular substance. The vacuolated cells are more numerous in the animals which have been under experimentation for the longest period of time. In the 15-month period, the vacuolated basophiles appear to be as numerous as the non-vacuolated type. The vacuolated cells become larger as the period of the experiment is lengthened. The complete loss of the testes causes the most hypertrophy of the vacuolated cells. However, these cells are found to be present in all of the experimental animals even though the interstitial tissue and some degenerate germinal epithelium are present.

The polarity of the Golgi material apparently is not of the importance which previous workers had attributed to it. In the present study, the Golgi material may be located on the side of the cytosome which is opposite the capillaries as frequently as it may be observed on the surface of the cytosome which borders the capillaries.

The Golgi material of the basophiles enlarges considerably following the loss of the germinal epithelium of the testes which is shown by figure 4. The most hypertrophy is produced by gonadectomy. The hypertrophy of the Golgi material is a continuous process which may be seen by studying, especially, the results of castration and of irradiation of the testes. The hypertrophy of the Golgi material reaches approximately the same level in all experiments except after gonadectomy, in which case it is greater. In all the other

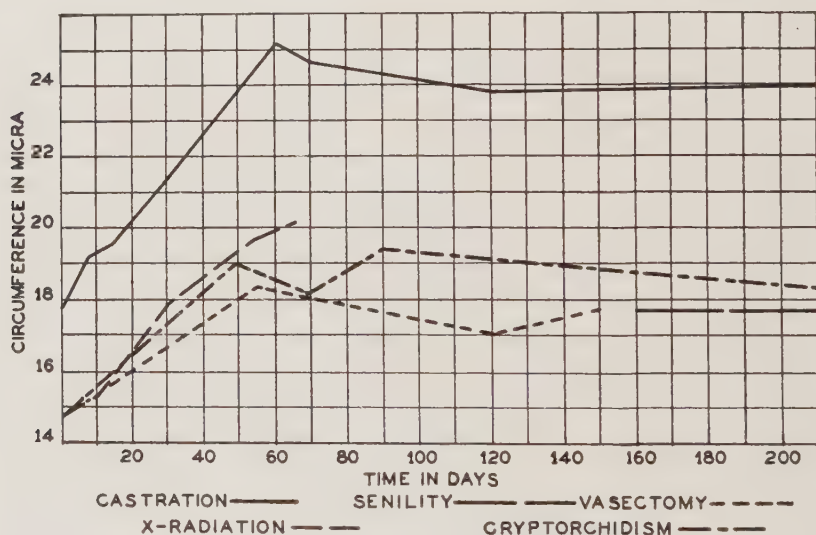


Fig. 4 Circumference of the Golgi material in the basophiles in the anterior lobe of the rat hypophysis.

cases the interstitial tissue and the germinal epithelium, which is degenerate, are present. The hypertrophy of the material then reaches the level of that following senility.

The Golgi material presents another series of modifications which are of great interest. In a recent paper the writer ('37) has shown the relationship between the secretory process of the basophilic cells and the Golgi material. The latter substance often exhibits an uneven periphery which is due to the formation of small secretory droplets in its osmicated margin, causing an evaginated appearance (figs. 12 and

15). In many instances granules or small secretory droplets, surrounded by an osmiophilic girdle, are present in the lighter central portion of the Golgi material (figs. 12 and 14). These structures are frequently found in the normal gland (fig. 13) but never are as numerous as they are in the experimental hypophyses.

All of the experimental animals showed basophiles in which the formation of some small vacuoles was occurring in the cytosome near the Golgi material. Some of the peripheral evaginations of the Golgi material containing secretion droplets may be observed along the periphery of the forming vacuole (fig. 17). Apparently the osmiophilic girdles, with their included secretion, move out from the Golgi material in the cytosome which surrounds the vacuole. Then a number of osmiophilic girdles, containing secretion droplets, may be observed along the margins of the newly forming vacuoles (fig. 17). These same structures are often located in a similar position in the large vacuolated cells (figs. 11 and 16).

Numerous osmicated granules and threads, which arose from the osmiophilic girdles when the secretion was liberated into the large vacuole, are often seen in the narrowed rim of the cytosome which borders the vacuole of the vacuolated basophile. The actual liberation of the secretion from the osmiophilic girdle was not observed. The fragmentation of the osmiophilic girdle is not caused by cytolysis nor is it due to post mortem changes since all erythrocytes in the sections presented regular borders.

The Golgi material of the vacuolated cell is not necessarily in the form of a distinct 'body.' Frequently the cells contain only osmiophilic girdles and fragments in the rim of cytoplasm surrounding the vacuole. In about 50% of the cells measured, the Golgi material was found in the form of a 'body' which was always located between the nucleus and the vacuole. The 'body' is usually not as large as in the non-vacuolated basophiles and frequently is somewhat flattened.

Figure 5 shows the size of the Golgi material in the vacuolated cells. It will be seen that the Golgi material increases in size up to about the second month and then decreases in size. The response of the gonadectomized animals is not as great up to the second month while the decrease is very marked. It will be seen that eventually the size of the 'body' becomes smaller than it is in the normal cell in all of the experiments. In the present instance, the effects of artificial cryptorchidism and vasectomy after 5 months are approximately the same. The size of the Golgi material in the above case is about that of the normal basophile.

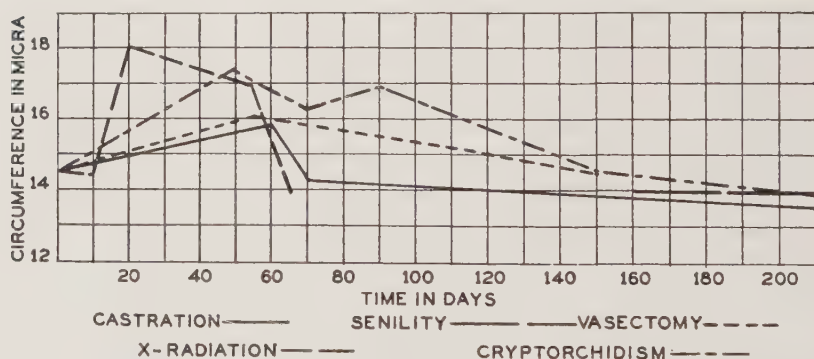


Fig.5 Circumference of the Golgi material in the vacuolated basophiles in the anterior lobe of the rat hypophysis.

DISCUSSION

The results of this experiment shows that the acidophiles of the anterior lobe of the hypophysis apparently have no role in the gonad-hypophyseal relationship. It will be remembered that the acidophiles and their contained Golgi material have decreased in size in all cases. The Golgi material also does not present any evidence of secretion.

The role of the chromophobe cells may be postulated from what is known concerning the normal development and function of the gland. The chromophobe cells, during the histogenesis and early development of the hypophysis, are continually being differentiated into the two types of the chromophilic

cells. The cell counts of Nukariya ('26) on rats, of Rasmussen ('29) on the human, and of Martins and de Mello ('35) on rats have shown that slightly more than 50% of the cells of the anterior lobe are chromophobes. The results of Martins and de Mello ('35), as well as others, have shown that the chromophobes decrease in number and the basophiles increase in number following gonadectomy and cryptorchidism. The loss of the normal germinal epithelium removes much of the inhibitory influence of the gonad on the hypophysis. As a consequence, the basophilic cells are increased in number. This constant differentiation affords an explanation for the appearance of the various sized basophiles which are observed in the glands of the experimental animals.

The basophiles are very sensitive to the loss of the germinal epithelium in the testes. The basophilic cells gradually increase in size up to the second month after which the curve shows a decrease in all cases. The Golgi material of the basophiles shows the same result. The basophilic cells, present in the hypophysis at the time of the loss of the germinal epithelium, increase in size immediately. At the same time numerous chromophobe cells are differentiating into basophiles. The appearance of these latter cells causes a decrease in the group size of the basophiles and their contained Golgi material. This supposition is further supported by the appearance of three distinct sizes of basophiles, when the cells are measured. The presence of the degenerate germinal epithelium and the interstitial cells provides some inhibitory effect on the hypophysis since the basophiles do not become as large following vasectomy, x-radiation, artificial cryptorchidism, and senility as they do following complete gonadectomy.

The basophilic cells also show an increased secretory activity which is shown by a study of the Golgi material. The osmophilic girdles which surround secretory droplets are much more numerous following the loss of the germinal epithelium than they are in the normal hypophysis. The seminiferous epithelium apparently controls the amount of hormone liberated by the basophiles. When the epithelium is

destroyed the basophilic cells show an overactive secretory cycle which is exhibited by the appearance of the numerous osmiophilic girdles containing secretion droplets. The fact that the basophiles show an increased secretory activity is further shown by the appearance of the vacuolated cells. The contents of the secretion droplets are of the same nature as that shown within the vacuoles. Furthermore the droplets eventually took up a position adjacent to the vacuole and the remnants of the osmiophilic girdles are seen scattered in the narrow rim of the cytosome which surrounds the vacuole.

The vacuole of the vacuolated basophile presumably functions as a reservoir retaining the excess secretion of the cell. It has previously been shown by a number of workers that the implanted hypophysis of a gonadectomized animal is three times more potent than the implanted hypophysis of the normal animal. Since the appearance of the vacuolated cell is the only major modification in the anterior lobe, it would appear that the substance which is stored in the vacuolated cell is of prime importance in the increased effectiveness of the hypophysis following gonadectomy.

The vacuolated basophilic cells, which I have shown arise in all male rats in which the germinal epithelium has been removed or is present in a degenerate condition, have previously been designated as the 'castration cells.' This is doubtless due to the work of Addison ('17) in which he proposed the term for the vacuolated cells which he observed in the pituitary following gonadectomy. From my results it appears that in the rat the cell might be more adequately designated as the 'sterility cell' since its appearance is not dependent on castration, but is dependent on the loss of the normal germinal epithelium which may be produced by numerous procedures other than castration; namely, artificial cryptorchidism, irradiation with x-rays, vasectomy, and senility (as well as by vitamin deficiency which was not attempted in this experiment).

SUMMARY

1. The acidophiles of the anterior lobe of the hypophysis of the albino rat show a progressive atrophy following the loss of the germinal epithelium from the testes. The atrophy affects the cytosome, nucleus and the Golgi material to the same degree.

2. The Golgi material of the acidophile, which is very compact and dense, does not undergo any variation in structure, density, or position following the loss of the germinal epithelium.

3. The chromophobes, seemingly, have the role of differentiating into the two types of chromophilic cells. There is no other apparent function.

4. The basophiles of the anterior hypophysis exhibit a progressive hypertrophy following the loss of the seminiferous epithelium from the testes. The hypertrophy affects the cytosome, the nucleus and the Golgi material.

5. The basophilic cells also increase in number following the loss of the germinal epithelium. The cells may be divided into three groups:

a. Small homogeneous cells which have the appearance of the basophiles found in the normal hypophysis. These cells have in all probability recently differentiated from the chromophobe cells.

b. Large cells which show numerous osmiophilic granules, and girdles either within the enlarged Golgi material or in the peripheral portion of the material. These structures demonstrate the beginning of an active secretory cycle in the large cells.

c. Large vacuolated cells which have originated from the basophilic cells. In my opinion, the term 'sterility cell' would be more appropriate than any other given since the cell prominently appears 2 months after the loss of the germinal epithelium. In these cells the Golgi material often assumes the form of discrete granular or thread-like particles, as well as the osmiophilic girdles, instead of the distinct 'body.' These cells demonstrate the termination of an over-active secretory

cycle. The vacuole of the cells usually contain a finely granular substance which is similar to that found in the secretory droplet. This group of cells may be regarded as storage cells of the gonadotropic substance which arises from the basophilic cells.

LITERATURE CITED

- ADDISON, WM. H. F. 1917 The cell changes in the hypophysis of the albino rat after castration. *J. Comp. Neur.*, vol. 28, p. 441.
- ATWELL, W. J. 1929 Characteristics of the Golgi apparatus in the different types of cells in the anterior lobe of the cat's hypophysis. *Anat. Rec.*, vol. 42, p. 44.
- 1932 Characteristics of the Golgi apparatus in the different types of cells of the anterior hypophysis. *Anat. Rec.*, vol. 55, p. 11.
- GATZ, A. J. 1933 Comparative cytological study of the anterior lobe of the hypophysis of male albino rats affected by gonadectomy. Thesis, Univ. of Minn.
- 1936 A study of the cytological relationship between the hypophysis and the germinal epithelium of the testis in the albino rat. Ph.D. thesis, Univ. of Minn.
- 1937 The relation of the Golgi material to the secretory process in the basophilic cells of the anterior hypophysis. *Anat. Rec.*, vol. 69, p. 429.
- GUYER, M. F. 1930 *Animal Micrology*. 3rd ed. The Univ. of Chicago Press. Chicago.
- GUYER, M. F., AND P. E. CLAUS 1932 Cell changes in the anterior lobe of the pituitary following cancer transplantation in rats. *Anat. Rec.*, vol. 52, p. 225.
- 1934 The Golgi apparatus in relation to vacuolation in the basophiles of the anterior pituitary of castrate and of cancerous rats. *Anat. Rec.*, vol. 61, p. 57.
- 1937 Vacuolation of the anterior pituitary gland following castration, implantation of cancer tissue and thyroidectomy. *Anat. Rec.*, vol. 67, p. 145.
- LEE, B. 1928 *Microtometist's Vade-Mecum*. 9th ed. P. Blakiston's Son and Co. Philadelphia.
- MARTINS, T., AND R. F. DE MELLO 1934 Pourcentage relatif des types cellulaires dans l'hypophyse antérieure des rats normaux et des rats cryptorchides. *Revisse de Biologia e Hygiène*, T. 5, p. 80.
- 1935 Pourcentage relatif des types cellulaires dans l'hypophyse antérieure des rats normaux et des rats cryptorchides. *C. R. de la Soc. de Biol.*, T. 118, p. 916.

- NUKARIYA, S. 1926 Ueber die Bedeutung der Rückresorption des Spermas (auf Grund von Spermainjektion an Kastraten) un über mikroskopische Veränderungen der Hypophyse an jungkastrierten weissen Ratten. Arch. f. d. Ges. physiol., Bd. 214, S. 697.
- RASMUSSEN, A. T. 1929 The percentage of the different types of cells in the male adult human hypophysis. Am. J. Path., vol. 5, p. 263.
- SEVERINGHAUS, A. E. 1933 A cytological study of the anterior pituitary of the rat with special reference to the Golgi apparatus and to cell relationship. Anat. Rec., vol. 57, p. 149.

PLATE 1

EXPLANATION OF FIGURES

6 Cross section of testis of normal animal. Notice the pronounced activity of the seminiferous epithelium and the amount of interstitial tissue present. Compare the size of the tubule with those of succeeding figures. $\times 162.5$.

7 Cross section of the testis of a rat 2 months after x-radiation of the testis. The normal germinal epithelium is replaced by a vacuolated fibrillar cytoplasm in which the nuclei are very scarce and pyknotic. The intertubular space is more prominent and filled with an accumulation of lymph and prominent interstitial cells. The tubules have decreased in size markedly. $\times 162.5$.

8 Cross section of the testis of a vasectomized animal. The normal germinal epithelium is being replaced by a vacuolated fibrillar cytoplasm which has pyknotic nuclei. The intertubular space is about as marked as in the other cases. It is filled by cords of interstitial cells and a lymphoid accumulation. The tubules are smaller than those found in the normal animal. $\times 162.5$.

9 Testis of an artificial cryptorchid rat. The tubules are lined by a vacuolated fibrillar cytoplasm containing pyknotic nuclei. The intertubular space is enlarged and contains lymphoid material and interstitial cells. $\times 162.5$.

10 Testis of a senile rat. The shrunken tubules present no normal germinal epithelium, but are filled with a degenerate cytoplasm. The intertubular space, which is increased in area, is filled with lymph and interstitial cells. $\times 162.5$.

11 Vacuolated basophile from the anterior lobe of the hypophysis of a rat which had been gonadectomized for 15 months. The cell is slightly out of focus so that numerous osmiophilic girdles with their contained droplets (OG) may be seen in the narrowed rim of the cytosome. (V) refers to the large vacuole filled with a finely granular substance. $\times 1100$.

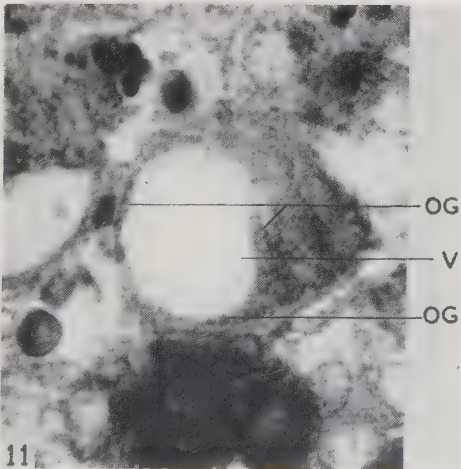
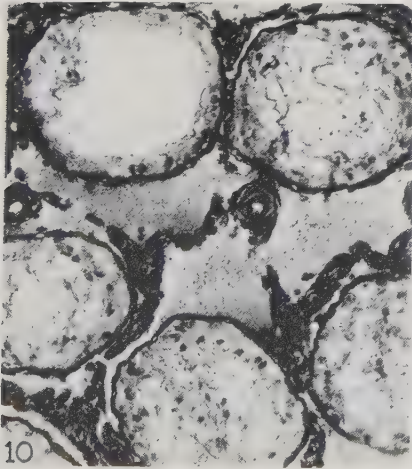
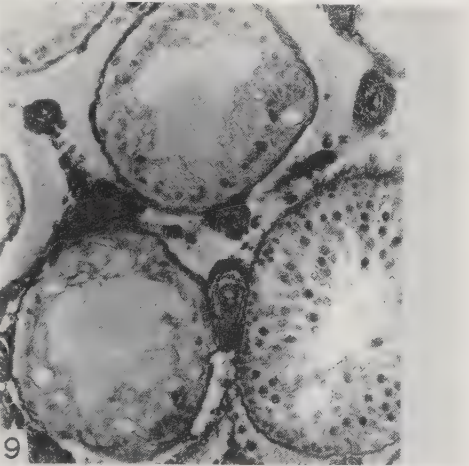
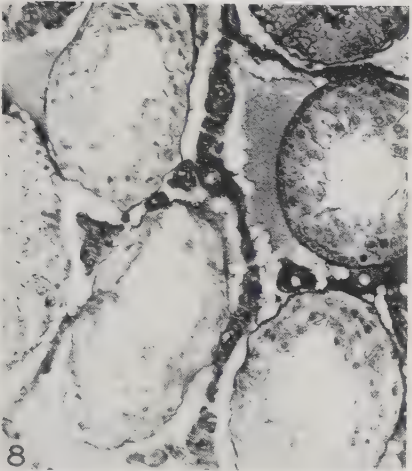
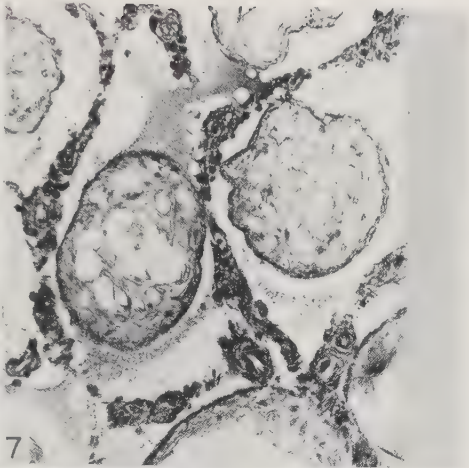


PLATE 2

EXPLANATION OF FIGURES

12 Basophile of the rat anterior lobe 15 months after gonadectomy. The nucleus and the Golgi material, which is considerably larger than the nucleus, are readily seen. The Golgi material contains numerous small osmiophilic girdles within its macula and osmicated periphery. At point X, the girdles are seen exuding from the periphery. X 885.

13 Basophile from the anterior hypophysis of a normal rat. The nucleus, which is not sharply defined, is larger than the Golgi material. Some osmiophilic girdles are seen in the periphery of the material. X 1100.

14 Basophile from the anterior lobe of the hypophysis 2 months after gonadectomy. The Golgi material and the nucleus are approximately the same size. The periphery of the Golgi material contains numerous osmiophilic girdles. X 1100.

15 Basophiles from the anterior hypophysis 2 months after the testes have been x-radiated. This figure demonstrates the cord-like distribution of the basophiles which is frequently seen. X 1100.

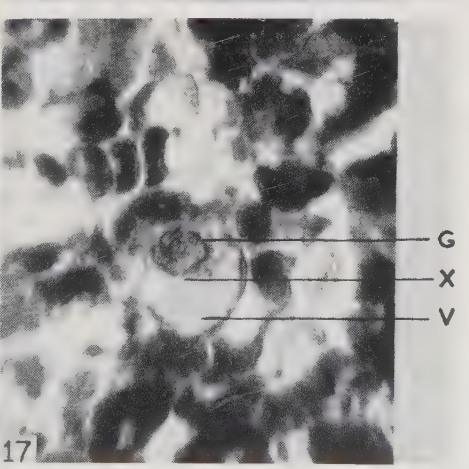
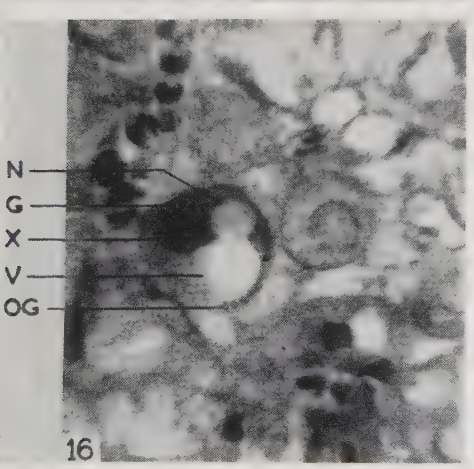
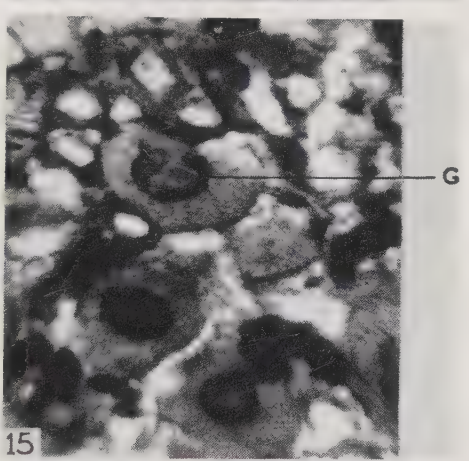
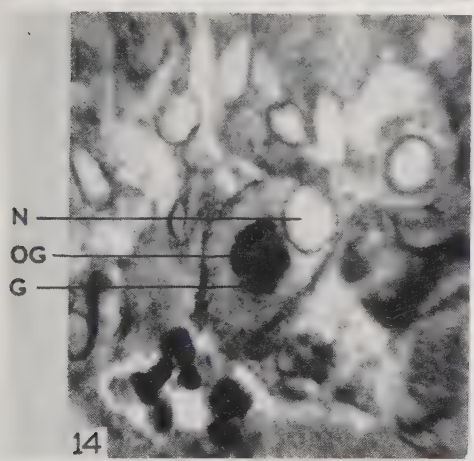
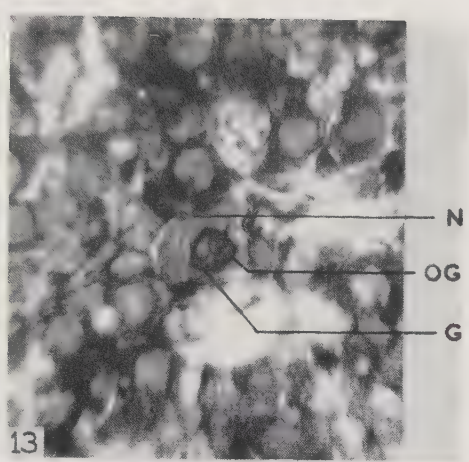
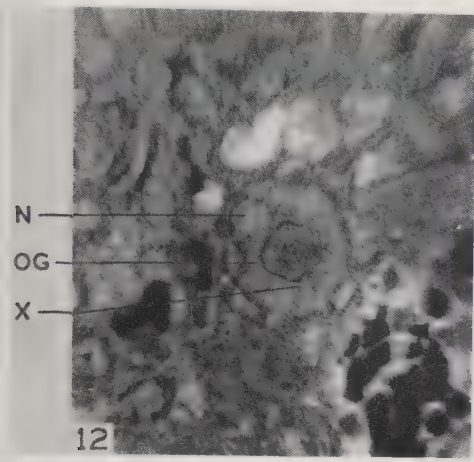
16 Small vacuolated basophile from the anterior lobe 2 months after gonadectomy. The nucleus and Golgi material are clearly shown. The narrowed rim of cytoplasm contains osmiophilic girdles. At point X the girdles are seen in close proximity to the vacuole. X 1100.

17 Small vacuolated basophile from the anterior lobe of the hypophysis 2 months after x-radiation of the testes. The Golgi material shows marked secretory activity. Numerous small girdles are seen lining the periphery of the vacuole at point X. The nucleus is not visible in this view. X 1100.

ABBREVIATIONS

G, Golgi material
N, nucleus

OG, osmiophilic girdle
V, vacuole



THE HOMOLOGY OF THE VESICULAR OVARIAN FOLLICLES OF THE MAMMALIAN OVARY WITH THE COELOM¹

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ONE TEXT FIGURE AND ONE PLATE (EIGHT FIGURES)

For many years the most widely accepted theory of the origin of the coelom has been the gonocoel theory. In this paper the problem considered is in a sense the reverse of this. It is the theory of the homology of the cavities of the ovarian follicles of mammals with the coelom, and it is largely based on the ontogenetic fact of the origin of the follicular epithelium from the peritoneal covering of the ovary, although certain other accessory evidence will be presented. In brief the evidence indicates that the follicular cavities of mammals are isolated portions of the coelom, and that the follicular epithelium and lutein cells are specialized portions of the coelomic epithelium. For convenience and clarity each supporting fact is discussed separately in the following six paragraphs.

1. Primordial ova occasionally form partly developed primary oocytes while still lying in the surface epithelium of the ovary or just within the mouth of an egg tube. This condition has been observed several times. It is especially well shown in the ovary of the porcupine (figs. 4, 5, 6, 7 and 8), and occasionally in the pocket gopher and the grizzly bear. These surface eggs sometimes enlarge considerably but eventually undergo hyalin degeneration (fig. 8). Clark ('23)

¹This work was aided by a grant from the Wisconsin Alumni Research Foundation.

described and figured ova in the germinal epithelium of a 3-month-old guinea pig. She suggested the possibility that these ova had migrated to the surface from the interior of the ovary, but thought this unlikely. In this she was surely correct, for in the porcupine early stages of differentiation of these ova from the surface epithelium are easily found (fig. 7). Such surface eggs might also be considered to have developed from residual primordial germ cells, but there is certainly no evidence of this and it seems much more probable that they differentiate from the generalized ovarian epithelium. The ones illustrated in figure 7 were in an animal at least 6 months old although not an adult. They have been found in adults, even pregnant animals, but less commonly than in the younger individuals. The presence of such surface eggs illustrates the generally accepted fact that the surface or germinal epithelium of the ovary contains cells which have the potentialities of germ cells. Of course this brings up the question of whether the epithelial covering of the ovary should be thought of as merely a specialized portion of the coelomic lining or as a distinctly different tissue, the 'germinal epithelium.' There is little in favor of the latter view, and so I shall consider the ovarian surface epithelium homologous to the peritoneum. One might carry the homology even farther and compare the tunica albuginea and theca interna and externa with the fibrous layer of the peritoneum.

2. Ovarian follicles in all stages from fetus to adult are derived from tubes or cords of cells invaginated from the surface epithelium of the ovary. These often retain their connection with the surface epithelium until after primordial ova and even primary follicles are developed within them. Figures 4 and 5 show the invagination of tubes from the surface epithelium in the porcupine ovary. Figure 2 shows similar tubes in the ovary of an adult grizzly bear, and figure 3 shows numerous more or less solid cords in the ovary of a young bitch. In most mammals these 'tubes' are really cords but that does not alter the fact that they are sprouts from the coelomic lining. This indicates that the follicular cavities

later developed in them may be regarded as homologous to the peritoneal cavity, since their lining cells are derived directly from a portion of the peritoneal epithelium.

3. The follicular epithelium is a serous epithelium like that of the peritoneum. It is true that the granulosa cells are not of the squamous type as are those of the general peritoneum, for they have retained their embryonic more-or-less columnar shape and also their embryonic capacity for mitosis. In the older follicles they form a stratified layer which is also not characteristic of coelomic epithelium in general. On the other hand in cystic follicles where, having lost their specialized function, they persist for months or years beyond their normal life span they do form a single layer of epithelial cells more characteristic of the adult peritoneum. Thus because of its derivation from the coelomic epithelium of the ovary and because of the embryonic nature of its cells the ovarian epithelium may be regarded as a portion of the coelomic lining serving a peculiar function as the lining of an egg-bearing follicle. For some reason, perhaps the necessity for rapid follicular growth, the persistence of the embryonic characteristics of the follicular epithelium is demanded. It may pass from this embryonic condition in either of three directions. 1) Normally, through follicular rupture to the formation of specialized endocrine cells, the lutein cells, and then eventually to degeneration. 2) Normally (in the sense that it is of universal occurrence) through follicular atresia to degeneration. 3) Abnormally, through loss of its special function, to form a cystic follicle where the epithelium seems to mature and become more like the normal, adult coelomic lining.

4. Follicular fluid is essentially a serous fluid although the albuminous material in it must be more concentrated than in ordinary peritoneal fluid. Studies of Hill, Allen and Kramer ('35), and McKenzie and Terrill ('37) on follicular rupture have shown that it may be in part gelatinous. The follicular hormone is found in it, but not in greater concentration than in extracts of the whole ovary, indicating that the hormone probably diffuses into the follicular fluid from the endocrine

cells of the theca interna and that its presence in the fluid is more or less fortuitous. To my knowledge there is no published account of the chemical nature of the follicular fluid, nor is there an analysis of normal peritoneal fluid of a mammal. Peters ('35) cites some work on the chemistry of ascitic fluid. The concensus of opinion seems to be that the coelomic fluid is formed from the blood serum by ultrafiltration and contains relatively little protein. There is no doubt that it contains some protein as it will coagulate quickly when a trace of blood is added. An accurate chemical analysis of peritoneal and follicular fluid from the same individual seems to be needed. Follicular rupture might possibly depend in part upon osmotic pressure due to passage of water from the peritoneal fluid to the undoubtedly more concentrated follicular liquor.

5. Ovarian follicles rupture into the peritoneal cavity at ovulation thus reestablishing connection with the coelom.

6. The corpus luteum in some species is occasionally everted to form a plaque on the ovarian surface so that the glandular tissue occupies the same relation to the coelom that its ancestral cells did before their invagination to form the egg tubes. This occurs frequently in the pocket gopher (fig. 9). The source of the new epithelium which covers the region of these everted corpora after they degenerate, or which overgrows the rupture points of normal corpora, is not known. It may regenerate from the normal epithelium surrounding the lutein area or rupture point, or may be differentiated directly from the mesenchymatous connective tissue cells which migrate into the cavity of the corpus luteum and which spread over the exposed surface of the rupture point or that of the everted corpus. My observations lead me to favor the latter process as the one of greatest importance, although undoubtedly there is some regeneration from the edges of the surrounding normal epithelium.

To summarize, it can be said that in mammalian ovaries the follicular epithelium is to be regarded as a modified coelomic epithelium, and the follicular cavities as isolated portions of

the peritoneal cavity filled with slightly modified peritoneal fluid. Figure 1 illustrates this conception of the follicle.

Such a relation of the follicle to the coelom can be traced phylogenetically as well as ontogenetically. While it is not the purpose here to discuss this phase of the problem brief mention may be made of certain facts. In practically all vertebrates the germinal epithelium of the ovary either lies entirely on the ovarian surface or develops at an early time from the

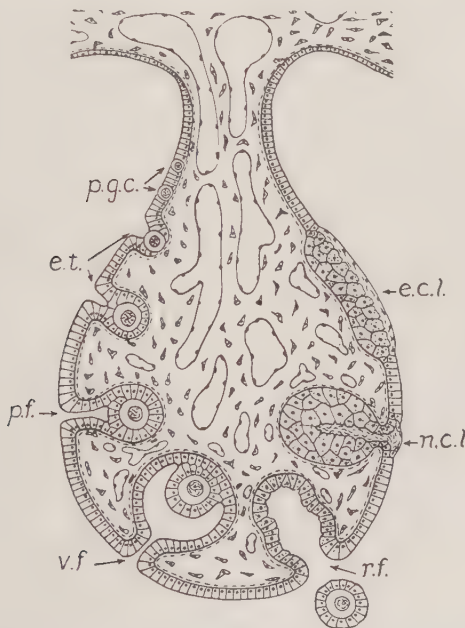


Fig. 1 Diagram of mammalian ovary to illustrate the theoretical homology of the follicular epithelium and lutein tissue to the coelomic lining and of the follicular cavity to the coelom. The only condition indicated on this diagram which has not been shown to occur normally is the connection of the cavity of a vesicular follicle by an open egg tube to the peritoneal cavity. In some animals a distinct, narrow prolongation of the follicular cavity toward the surface is indicative of this relationship. e.c.l., everted corpus luteum with fibroblastic layer covering it which would ordinarily be enclosed within the corpus except in the region of the healing rupture point (compare fig. 9). e.t., egg tubes. n.c.l., normal corpus luteum with cavity filled by fibroblastic tissue which is taking part in the healing of the rupture point. p.f., primary follicle connected to surface by egg tube. p.g.c., primordial germ cells or surface ova (compare figs. 4, 6, 7 and 8). r.f., ruptured follicle immediately after ovulation. v.f., vesicular follicle.

surface epithelium. The latter is by far the more usual condition. Also in practically all vertebrates the eggs develop either in the surface epithelium or in the deeper tissues, and upon ripening pass from, or through, the ovarian surface into the general body coelom or a cavity that is obviously a specialized portion of it (certain teleosts). These facts and similar ones were used long ago in developing the 'gonocoel' theory of the origin of the coelom. Hatschek (1878) is usually given credit for having started this line of reasoning when he suggested that the 'pole cells' which form the coelomic lining of annelids and molluscs might be primitive germ cells. However Bergh in 1885 on the basis of studies on the gonads of flat-worms first put the theory to the forefront as an explanation of the phylogenetic origin of the coelom throughout the animal kingdom. The enterocoel theory, first suggested by the work of Leuckart (1848) on coelenterates; Agassiz (1864) on echinoderms; Metschnikoff (1869) on echinoderms and *Balanoglossus*, was actually introduced as a theory of development of the coelom by Metschnikoff (1874). Lankester (1875) also proposed the idea of an enterocoel in connection with his 'planula' theory and elaborated upon it greatly later (1877). Huxley (1875) accepted the enterocoel theory for a few phyla, including echinoderms, *Sagitta* and *Balanoglossus*, but introduced the idea of a 'schizocoel' for molluscs and annelids, and of an 'epicoel' for tunicates and possibly vertebrates. The conception of the enterocoelous origin of the coelom was in general accepted by Balfour (1880) and by O. and R. Hertwig (1882). Therefore the rapid rise in favor of Bergh's 'gonocoel theory' after he introduced it in 1885, in spite of an impressive array of 'enterocoelists,' was testimony to its value. It was elaborated by Goodrich (1895), by E. Meyer ('01) and by Lang ('04). The latter made it the basis for his 'hemocoel' theory of the vascular system, and Goodrich ('30) still accepts it as the most logical and useful theory of phylogenetic origin of the coelom. The 'nephrocoel' theory introduced by Ziegler (1898) has never gained wide acceptance.

There are some facts on the basis of which the enterocoel, gonocoel and nephrocoel theories may be partially harmonized. This is particularly true of the first two. The 'enterocoel' theory was based on such forms as *Amphioxus* where the coelomic pouches are apparently evaginations of the archenteron. Even in annelids the pole cells temporarily form part of the lining of the archenteron and in coelenterates the gonads are definitely pouches of the enteron. The homology of the coelomic cavities of *Amphioxus* with those of higher chordates is reasonably clear, and in all the higher vertebrates, from fishes up, the germ cells seem to arise from, or at least to lie in, the coelomic lining, thus strongly indicating a very close phylogenetic relation between the gonads and the coelom.

Of course if one looks at the serous membranes from the functional standpoint most of the evidence, at least from the vertebrates, seems to point to their being adaptations within the mesoderm to allow freedom of movement between parts. Such an explanation would explain the early development of the pericardial cavity and the relatively late differentiation of the pleural cavities in vertebrate embryos. It would also explain other functionally similar cavities such as the synovial cavities of movable joints and the subcutaneous synovial bursae of the human body as well as the even more beautifully adapted synovial tendon sheathes. Wood-Jones ('13) in his discussion of the functional history of the coelom considers this and several other functions in detail.

A somewhat similar teleological explanation for the separation of the ovogenic coelomic epithelium from the general cavity may also be advanced. May it not be that the obvious disadvantages of the exposed coelomic lining as a site for the development of eggs led by adaptation to the carrying of this process beneath the ovarian surface? In such an environment the delicate ova may develop to full size and have a relatively tough vitelline membrane, or zona pellucida in the case of mammals, formed about them before they are finally thrown into the body cavity. Perhaps the mammalian fol-

licular antrum can also be explained as a necessary adaptation because of the small size of the mammalian egg. It is difficult to see how such a small particle could be set free with sureness, even though it developed on the surface of the ovary, if it did not have the body of follicular liquor to wash it out into the funnel of the oviduct. Such explanations however are of little scientific value, merely giving some of us a certain amount of mental satisfaction.

Whatever one proposes in regard to the fundamental structure and evolution of the ovary should also be applicable in a broad way at least to the testis. The male and female germ cells are certainly homologous. In mammals both testis and ovary pass through an indifferent stage which consists essentially of a mass of epithelial cords or tubules derived from the surface epithelium and separated by so-called 'intermediate' tissue probably of a mesenchymal instead of mesothelial origin. Thus the seminiferous tubules, and part at least of the ductuli efferentes are derivatives of the coelomic epithelium. Certainly on the basis of ontogenetic evidence we can go still further and consider the mesonephric tubules and duct as also mesothelial in origin, but this takes us into a consideration of the phylogeny of these structures which is entirely beyond the purpose of this paper.

However there is still one highly debatable point. Are we justified in making the assumption that the follicular antra of mammalian ovaries are either phylogenetically or ontogenetically derived from the coelom, or should we simply say that they are homologous to the coelom? There is no good reason to doubt that the follicular epithelium is both phylogenetically and ontogenetically derived from the coelomic lining. However, the cavities of the egg tubes are not simply broken into segments and then enlarged to form the antra of the follicles. Instead they disappear at the time the primary follicles are formed, and no follicular cavity is present until sometime later when the cells of the follicular epithelium separate again giving rise to the early antra. The phylogenetic history of the follicular cavity seems to be essentially

similar. That is to say, although egg tubes or cords form from the surface epithelium in many lower vertebrates, the follicles never retain or develop cavities, but have throughout their growth and maturity essentially the structure of a primary follicle of a mammalian ovary, except that the oocyte is larger because of its greater accumulation of yolk. This condition is equally true of the egg-laying mammals as well as of the immediate ancestral group, the reptiles. Therefore, both the ontogenetic and phylogenetic evidence seems to point to the ovarian follicles of mammals as a secondary cavitation in coelomic epithelium homologous to the coelomic cavity but not directly derived from it either during development of the individual or the race.

SUMMARY

The theory is proposed that the follicular epithelium and lutein cells of mammalian ovaries are both ontogenetically and phylogenetically specialized portions of the coelomic epithelium, and that the follicular cavities are homologous to the coelom although both their ontogenetic and phylogenetic histories indicate that they are not direct outpouchings of the coelom but develop secondarily, probably to meet the specialized conditions attendant upon the ovulation of the microscopically small mammalian ova.

LITERATURE CITED

- AGASSIZ, LOUIS 1864 Embryology of the starfish. *Contrib. Nat. Hist. U. S.*, vol. 5.
- BALFOUR, F. M. 1880 On the structure and homologies of the germinal layers of the embryo. *Quart. J. Mic. Sci.*, vol. 20, pp. 247-273.
- BERGH, R. S. 1885 Die Exkretionsorgane der Würmer. Eine Uebersicht. *Kosmos. Zeitschr. f. d. ges. Entwicklungslehre*, Bd. 17, S. 97-122.
- CLARK, ESTHER B. 1923 Observations on the ova and ovaries of the guinea pig, *Cavia cobaya*. *Anat. Rec.*, vol. 25, pp. 313-331.
- GOODRICH, E. S. 1895 Coelom, genital ducts and nephridia. *Quart. J. Mic. Sci.*, vol. 37, pp. 477-510.
- 1930 Studies in the structure and development of vertebrates, Macmillan, London, pp. 715-719.
- HATSCHKE, B. 1878 Studien über Entwicklungsgeschichte der Anneliden. Ein Beitrag zur Morphologie der Bilaterien. *Arbeit. Zool. Inst. Wien*, vol. 1.

- HERTWIG, O., AND R. HERTWIG 1882 Die Cölomtheorie. Versuch einer Erklärung des mittleren Keimblattes. *Zeitschr. Naturwiss.*, Bd. 15, S. 1-150.
- HILL, R. T., EDGAR ALLEN AND T. C. KRAMER 1935 Cinemicrographie studies of rabbit ovulation. *Anat. Rec.*, vol. 63, pp. 239-245.
- HUXLEY, THOMAS 1875 On the classification of the animal kingdom. *Quart. J. Mic. Sci.*, vol. 15, pp. 52-56.
- LANG, A. 1904 Beiträge zu einer Trophocöltheorie. *Zeitsch. f. Naturwiss.*, Bd. 38, S. 1-376.
- LANKESTER, E. RAY 1875 On the invaginate planula of *Paludina*. *Quart. J. Mic. Sci.*, vol. 15, pp. 159-166.
- 1877 Notes on the embryology and classification of the animal kingdom: comprising a revision of the speculations relative to the origin and significance of the germ layers. *Quart. J. Mic. Sci.*, vol. 17, pp. 399-454.
- LEUCKART, R. 1848 Ueber die Morphologie und Verwandtschaftsverhältnisse der wirbellosen Thiere. Ein Beitrag zur Charakteristik und Classification der tierischen Formen. Braunschweig.
- MCKENZIE, FRED F., AND CLAIRE E. TERRILL 1937 Estrus, ovulation and related phenomena in the ewe. *Missouri Agric. Exp. Sta. Bull.*, no. 264, pp. 1-88.
- METSCHNIKOFF, ELIAS 1869 Studien über die Entwicklung der Echinodermes und Nemertinen. *Mémoires de l'Acad. impériale d. sci. de St. Petersburg*. 7th ser., vol. 14, no. 8.
- 1874 Studien über die Entwicklung der Medusen und Siphonophoren. *Zeitschr. f. wissenschaft. Zool.*, Bd. 24, S. 15-83.
- MEYER, EDOUARD 1901 Studien über Körperbau der Anneliden. *Mittheilungen Zool. Station Neapel*, vol. 14, pp. 247-585.
- PETERS, JOHN P. 1935 Body Water. *The Exchange of Fluids in Man*. Thomas. Springfield and Baltimore.
- WOOD-JONES, FREDERIC 1913 The functional history of the coelom and the diaphragm. *J. Anat. and Physiol.*, vol. 47, pp. 282-318.
- ZIEGLER, H. E. 1898 Ueber den derzeitigen Stand der Cölomfrage. *Verhandl. der Deutschen Zool. Gesellsch.*, Bd. 8, S. 14-78.

PLATE

PLATE 1

EXPLANATION OF FIGURES

2 Egg tubes in the ovary of the adult grizzly bear. It can be shown by following the series of sections that the two primary follicles at the left are connected by their epithelium to the egg tube leading toward them from the ovarian surface directly above. *Ursus horribilis* ♀1, slide 2, section 3, 10 μ . $\times 50$.

3 Egg cords in the ovary of a nearly full grown but sexually immature bitch. Such follicles as the largest one (in this vase biovular), as well as the smaller ones above, can be shown to be connected to egg cords which occasionally may be traced all the way to the surface. The cords in the dog ovary are much more tortuous than in the bear. Longitudinal sections of the cords often show a row of three or more ova in them. *Canis familiaris* ♀4, slide 7, section 3, 10 μ . $\times 110$.

4 Surface and cortical portion of the ovary of an immature porcupine, probably about 6 months old, showing egg tubes, and surface eggs developing in the epithelium. *Erethizon dorsatus* ♀2, slide C₅, section 4, 10 μ . $\times 98$.

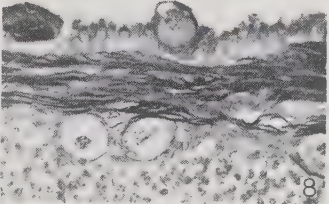
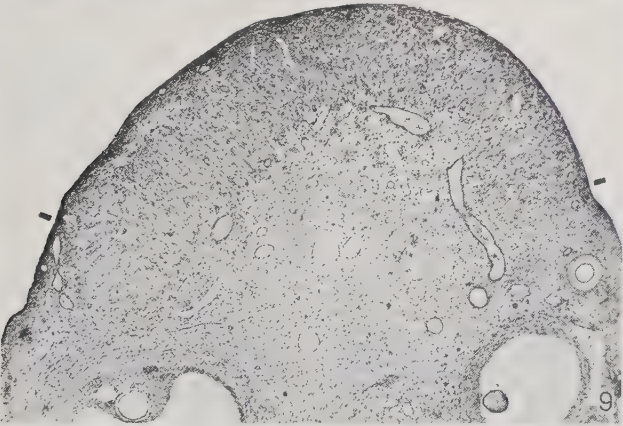
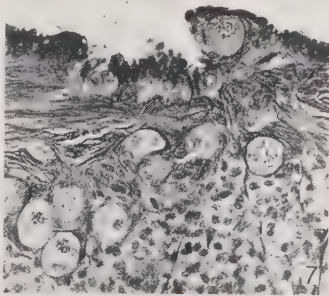
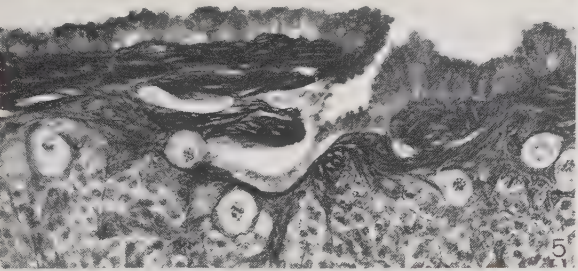
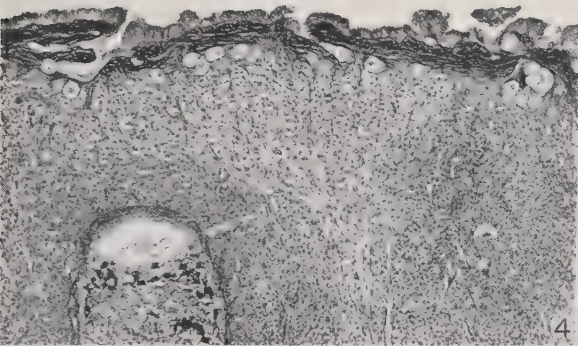
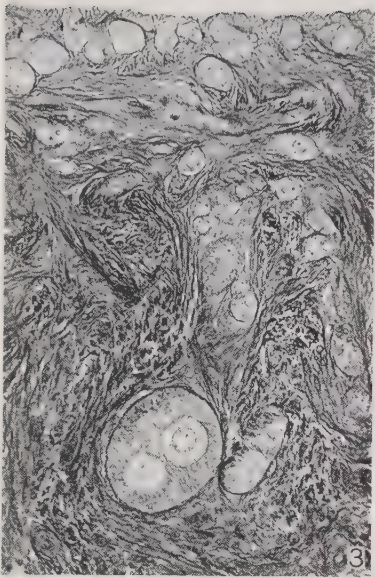
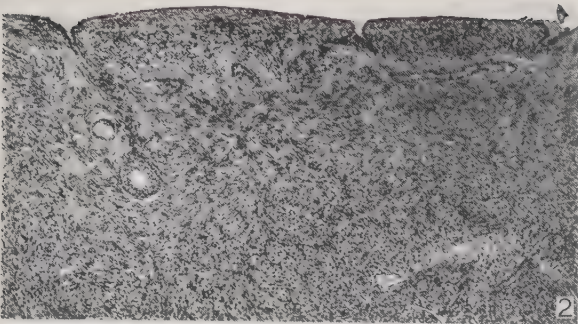
5 High power view of ovary shown in figure 4 showing detail of the egg tube penetrating the dense tunica albuginea. *Erethizon dorsatus* ♀2, slide C₅, section 4, 10 μ . $\times 250$.

6 High power view of ovary shown in figure 4 showing at the left the detail of a surface egg and an egg nest immediately deep to the tunica albuginea probably derived from an egg tube or cord which penetrated the tunic in that region. At the right are two ova undergoing enlargement between the fibers of the tunic. At the bottom of the indentation of the epithelium near the center are two lightly stained cells which are probably early stages in the differentiation of surface eggs from the epithelium. *Erethizon dorsatus* ♀2, slide C₅, section 4, 10 μ . $\times 250$.

7 High power view of ovary shown in figure 4 showing a surface egg and several enlarged and more lightly stained surface epithelial cells which seem to be in the process of differentiating into ova. *Erethizon dorsatus* ♀2, slide C₄, section 5, 10 μ . $\times 250$.

8 High power view of ovary shown in figure 4 showing surface egg with its thin capsule of flattened epithelial cells. The appearance of this egg suggests that it is being extruded from the epithelium. In the epithelium at the extreme left is a dark hyalin mass characteristic of the later stages of degeneration of these ova. *Erethizon dorsatus* ♀2, slide C₅, section 1, 10 μ . $\times 250$.

9 Everted corpus luteum in the ovary of a pocket gopher (*Geomys bursarius*). The interior of a normal corpus is represented by the fibroblastic tissue covering the convex surface of the ovary between the two marks. The outer surface of a normal corpus is represented by the inner concave boundary of the lutein tissue. Embryos in the early embryonic disc stage were just implanted in the uterus of this animal. *Geomys bursarius* ♀7a, slide 10, section 33, 8 μ . $\times 45$.



BOOK REVIEW

THE DEVELOPMENT OF THE VERTEBRATE SKULL. By G. R. de Beer. New York. Oxford University Press, 1937. xxiv + 552 pp. + 143 plates. \$10.00.

The appearance of the present volume is an event which all students of the vertebrates will, no doubt, celebrate in their own various ways as soon as they have made its acquaintance. The purpose of the author in publishing the work has been to make conveniently available all the information relating to the development of the skull in all the groups of the vertebrates, not solely that the information shall be available, but also that the lacunae in our knowledge be clearly exhibited and the reader stimulated to fill them by further investigation. It may at once be said that in this work Doctor de Beer has written what is unquestionably the best systematic description of the development of the skull in the various groups of vertebrates that has so far appeared. In fact, there exists nothing quite comparable to this work in any language.

Each vertebrate group is separately treated by the description of its representative types according to a uniform system of nomenclature. By this means it is possible to refer to the description of each form as a self-contained account, and to compare separate forms without having to resort to the necessity of a calculus of comparable terms, in short, without confusion. The use of a morphologically sound system of nomenclature is one of the excellent features of the work. In each group the first form receives a detailed description which serves as a type for the group as a whole, it being then possible to describe the following forms more summarily. At the end of the book there are some 700 excellently done figures, all drawn and lettered according to a uniform scheme, which clearly illustrate the various morphologic and developmental features referred to in the text.

The Introductory Section opens with an historical account of the subject with which the book deals. The Segmental Composition of the Skull and The Craniogenic Materials are then discussed in the two following sections. In the Systematic Section the various aspects of the development of the skull in all the vertebrate groups are described and discussed, and in the Comparative Section there is a

General Morphological Consideration of Certain Cartilages, and a General Consideration of Certain Bones.

In the General Section of the work the materials of the preceding sections are dealt with in the following divisions: Embryology and Evolution of the Skull, The Phylogeny of the Chondrocranium, The Growth of the Skull, Causal Relationships in the Development of the Skull, General Morphological Considerations. In an agendum the author states a fairly large number of problems which remain unanswered, and which he hopes will draw the interest of some investigators toward their solution. There is a model Bibliography and Index of Authors, an Index of Subjects, and finally an Index of Genera Referred to in the Systematic Section. Altogether this is a model treatise.

M. F. ASHLEY-MONTAGU
New York University

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SUPPLEMENT

AMERICAN SOCIETY OF ZOÖLOGISTS

Officers for 1937

<i>President</i>	F. L. HISAW
<i>Vice-President</i>	HELEN D. KING
<i>Secretary</i>	E. G. BUTLER
<i>Treasurer</i>	WALTER N. HESS

Executive Committee

(In addition to the four officers)

W. C. CURTIS.....	to serve until December, 1937
CHARLES ZELNY.....	to serve until December, 1938
A. H. STURTEVANT.....	to serve until December, 1939
ROBERT W. HEGNER.....	to serve until December, 1940
W. C. ALLEE.....	to serve until December, 1941

*Representative of the Society in the Division of Biology and Agriculture of the
National Research Council*

To serve until July 1, 1938.....	L. V. HEILBRUNN
	C. R. MOORE, Alternate

*Representatives of the Society on the Council of the American Association for the
Advancement of Science*

D. E. MINNICH

B. H. WILLIER

*Representatives of the Society on the Council of the Union of American
Biological Societies*

H. E. JORDAN

H. B. GOODRICH

*Representatives of the Society on the Advisory Editorial Board of
"Biological Abstracts"*

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D. H. TENNENT.....	<i>General Embryology</i>
M. H. JACOBS.....	<i>General Physiology</i>

*Representative on the Executive Committee of the Commission for the
Standardization of Biological Stains*

S. I. KORNHAUSER

Managing Editor of the Journal of Morphology

To serve until December, 1941.....	C. E. McCLUNG
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Members of Editorial Board of the Journal of Morphology

To serve until December, 1937

D. H. TENNENT L. C. DUNN C. G. HARTMAN

To serve until December, 1938

SALLY HUGHES-SCHRADER E. E. JUST D. H. WENRICH

To serve until December, 1939

LEIGH HOADLEY W. A. KEPNER J. PERCY MOORE

AMERICAN SOCIETY OF ZOÖLOGISTS

CONSTITUTION

ARTICLE I

NAME AND OBJECT

Section 1. The Society shall be called the "American Society of Zoölogists."

Sec. 2. The object of the Society shall be the association of workers in the field of zoölogy for the presentation and discussion of new or important facts and problems in that science and for the adoption of such measures as shall tend to the advancement of zoölogical science.

ARTICLE II

MEMBERSHIP

Section 1. The membership of the Society shall consist of two classes:

a. Associate membership open to all actively engaged in the field of zoölogy who have had training in zoölogy equivalent to that required for the doctorate degree.

b. Active membership open to those who hold the doctor's degree in zoölogy or who have done work equivalent thereto, and who, in addition to the usual requirements for this degree, have published the results of substantial zoölogical research.

Sec. 2. Election to membership in the Society shall be upon recommendation of the Executive Committee.

Sec. 3. Associate members shall not hold office or vote, but may present papers before the Society without introduction. Associate members shall receive all literature issued by the Society, including the proceedings of the Society, and shall have the same privileges as active members in journal subscriptions.

Sec. 4. Associate members may be elected to active membership in the usual way when the research requirements of active membership have been met.

ARTICLE III

OFFICERS

Section 1. The officers of the Society shall be a President, a Vice-President, a Secretary, a Treasurer, and the members at large of the Executive Committee.

Sec. 2. The Executive Committee shall consist of the President, the Vice-President, the Secretary, the Treasurer, and five members elected from the Society at large. Of these five members, one shall be elected each year to serve five years. If any member at large shall be elected to any other office, a member at large shall be elected at once to serve the remainder of his term.

Sec. 3. These officers shall be elected by ballot at the annual meeting of the Society and their official terms shall commence with the close of the annual meeting, except that the Secretary and the Treasurer shall be elected triennially and shall serve for three years.

Sec. 4. The officers named in Section 1 shall discharge the duties usually assigned to their respective offices. (See by-laws.)

Sec. 5. Vacancies in the board of officers, occurring from any cause, may be filled by election by ballot at any meeting of the Society. A vacancy in either the Secretaryship or the Treasurership occurring in the interval of the meetings of the Society may be filled, until the next annual meeting, by appointment by the Executive Committee.

Sec. 6. At the annual meeting the President shall name a nominating committee consisting of three active members. It shall be the duty of this committee to nominate officers of the Society, including the permanent representatives which the Society is asked to name to various boards and councils. This committee shall make its nominations to the Secretary at least four months before the next annual meeting. Additional nominations for any office may be made in writing to the Secretary by any five members at any time previous to balloting.

ARTICLE IV

MEETINGS OF THE SOCIETY

Section 1. Unless previously determined by the Society, the time and place of the annual meeting shall be determined by the Executive Committee. Special meetings may be called and arranged for by the Executive Committee. Notices of such meetings shall be mailed to all members of the Society at least two weeks before the date set for the meeting.

Sec. 2. Sections of the Society may be organized in any locality by not less than ten members, for the purpose of holding meetings for the presentation of scientific papers. Such sections shall have the right to elect their own officers and also associate members; provided, however, that associate membership in any section shall not confer membership in the Society.

ARTICLE V

QUORUM

Twenty-five members shall constitute a quorum of the Society and four a quorum of the Executive Committee.

ARTICLE VI

CHANGES IN THE CONSTITUTION

Amendments to this Constitution may be adopted at any meeting of the Society upon approval of two-thirds of the members voting, subject to the following conditions:

a. The proposed amendment must be in writing and signed by at least five members of the Society.

b. This signed proposal must be in the hands of the Secretary at least one month before the meeting of the Society at which it is to be considered.

c. The Secretary shall mail copies of the proposed amendment to the members of the Society at least two weeks before the meeting.

d. Members unable to attend may submit their votes in writing to the Secretary.

BY-LAWS

ARTICLE I

DUES

Section 1. The annual dues for active and associate members, unless remitted or changed by the vote of the Society, shall be two dollars.

Sec. 2. Any member in arrears of dues for two years should be dropped by the Executive Committee. Upon payment of arrearage he shall be reinstated.

ARTICLE II

PRESIDENT

Section 1. The President shall preside at scientific sessions and at the business meetings.

Sec. 2. He shall also appoint the committees prescribed by the constitution and by-laws.

ARTICLE III

VICE-PRESIDENT

Section 1. The Vice-President shall preside at sessions designated by the President.

Sec. 2. He shall also assume the duties of the President in the latter's absence.

ARTICLE IV

SECRETARY

Section 1. The Secretary shall keep the records of the Society.

Sec. 2. He shall be responsible for all arrangements for the meetings, including the appointment of local committees.

Sec. 3. Whenever the proper officers of a number of related societies shall have a conference covering matters of mutual interest to the several societies, he shall act as the delegate or representative of this Society.

Sec. 4. He shall make such purchases and employ such assistance as will, in his judgment, expedite the business of the Society, and

Sec. 5. He shall be reimbursed out of the funds of the Society for expenses incurred in attending meetings of the Society.

ARTICLE V

TREASURER

Section 1. The Treasurer shall be in charge of the funds of the Society.

Sec. 2. At the annual business meeting of the Society he shall present a statement to date of the funds of the Society.

Sec. 3. He shall make such purchases and employ such assistance as will, in his judgment, expedite the business of the Society.

ARTICLE VI

AUDITING COMMITTEE

The President shall annually appoint an auditing committee of two, who shall audit and report upon the financial record and statement of the Treasurer at the meeting for which they were appointed.

ARTICLE VII

PROGRAM RULES

In matters relating to programs for annual meetings, the following rules shall be observed:

Section 1. Titles and abstracts of papers to be presented by any method at the annual meeting must be sent to the Secretary in duplicate before the final date set by him. The length, form, and arrangement of the abstracts must comply with the requests of the Secretary in order to insure prompt publication by The Wistar Institute. The titles and abstracts of papers which do not conform to such requests or papers which are received late may be presented by title only.

Sec. 2. Titles shall be listed in groups according to subject matter, the groups to be determined by the Secretary.

Sec. 3. Whenever conditions require, the Secretary shall schedule two or more groups for the same hour and arrange the program to bring together papers on subjects of more general interest for meetings of the whole Society.

Sec. 4. Each member may have not more than fifteen minutes of the time of the Society at the annual meeting. This time may be used by him to present one or more papers personally or to introduce papers of non-members.

Sec. 5. All papers not read when called for as listed shall be placed at the end of the group list, and if not read when called for the second time, shall be read by title only.

Sec. 6. The titles of 'introduced' papers may be listed in the groups after the titles of papers to be read by members.

LIST OF FORMER OFFICERS

AMERICAN MORPHOLOGICAL SOCIETY

<i>President</i>	<i>Vice-President</i>	<i>Secretary-Treasurer</i>
1890—E. B. Wilson		J. P. McMurrich
1891—C. O. Whitman	E. L. Mark	J. P. McMurrich
1892—C. O. Whitman	H. F. Osborn	J. P. McMurrich
1893—C. O. Whitman	E. B. Wilson	J. P. McMurrich
1894—C. O. Whitman	W. B. Scott	G. H. Parker
1895—E. B. Wilson	W. B. Scott	G. H. Parker
1896—E. L. Mark	H. F. Osborn	G. H. Parker
1897—C. S. Minot	S. I. Smith	G. H. Parker
1898—H. F. Osborn	T. H. Morgan	G. H. Parker
1899—E. G. Conklin	W. M. Wheeler	Bashford Dean
1900—T. H. Morgan	H. C. Bumpus	J. S. Kingsley
1901—J. S. Kingsley	E. A. Andrews	T. H. Montgomery
1902—H. C. Bumpus	G. H. Parker	M. M. Metcalf

Additional Members of the Executive Committee

1891—E. B. Wilson	1897—J. S. Kingsley
H. F. Osborn	Bashford Dean
1892—E. L. Mark	1898—C. B. Davenport
T. H. Morgan	F. R. Lillie
1893—T. H. Morgan	1899—J. P. McMurrich
C. B. Davenport	G. H. Parker
1894—E. A. Andrews	1900—F. R. Lillie
F. H. Herrick	Jacob Reighard
1895—T. H. Morgan	1901—C. F. W. McClure
S. Watase	C. W. Hargitt
1896—E. G. Conklin	1902—H. S. Jennings
William Patten	R. G. Harrison

AMERICAN SOCIETY OF ZOÖLOGISTS

<i>EASTERN BRANCH</i>	<i>President</i>	<i>CENTRAL BRANCH</i>
G. H. Parker	1903	Jacob Reighard
E. A. Andrews	1904	C. H. Eigenmann
W. E. Castle	1905	F. R. Lillie
W. E. Castle	1906	C. C. Nutting
C. B. Davenport	1907	S. A. Forbes
W. M. Wheeler	1908	E. A. Birge
H. S. Jennings	1909	E. A. Birge
T. H. Montgomery	1910	C. E. McClung
H. V. Wilson	1911	George Lefevre
A. G. Mayer	1912	H. B. Ward
Raymond Pearl	1913	H. B. Ward

LIST OF FORMER OFFICERS

EASTERN BRANCH

Jacob Reighard
W. E. Castle
William Patten
William Patten
F. H. Herrick
H. S. Jennings
H. V. Wilson
H. H. Wilder
H. E. Crampton
G. A. Drew
Alex. Petrunkevitch

Vice-President

1903
1904
1905
1906
1907
1908
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1910
1911
1912
1913

CENTRAL BRANCH

H. F. Nachtrieb
S. J. Holmes
William A. Locy
George Lefevre
H. B. Ward
M. F. Guyer
M. F. Guyer
H. F. Nachtrieb
R. H. Walcott
C. M. Child
C. M. Child

Secretary-Treasurer

G. A. Drew
G. A. Drew
H. S. Pratt
H. S. Pratt
C. J. Herrick
L. L. Woodruff
L. L. Woodruff
H. W. Rand
Raymond Pearl
J. H. Gerould
Caswell Grave

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1912
1913

Frank Smith
F. R. Lillie
C. E. McClung
T. G. Lee
T. G. Lee
T. G. Lee
Charles Zeleny
H. V. Neal
H. V. Neal
W. C. Curtis
W. C. Curtis

Executive Committeemen

F. R. Lillie
T. H. Montgomery
H. C. Bumpus
H. S. Jennings
E. A. Andrews
W. R. Coe
G. A. Drew
M. M. Metcalf
D. H. Tennent
R. G. Harrison
H. E. Jordan
C. E. McClung
George Lefevre
T. G. Lee

Herbert Osborn
C. H. Eigenmann
J. G. Needham
S. J. Holmes
W. A. Locy
C. M. Child
R. H. Walcott
W. C. Curtis
Oscar Riddle
H. B. Ward
Chauncey Juday
H. W. Norris
C. E. McClung
H. F. Nachtrieb

AMERICAN SOCIETY OF ZOÖLOGISTS (AMALGAMATED)

<i>President</i>	<i>Vice-President</i>	<i>Executive Committeemen</i>
1914. C. E. McClung	M. F. Guyer	H. E. Jordan—1 year
1915. W. A. Locy	W. E. Ritter	H. F. Nachtrieb—2 years
1916. D. H. Tennent	Charles Zeleny	H. V. Wilson—3 years
1917. M. M. Metcalf	Charles Zeleny	George Lefevre—4 years
1918. George Lefevre	L. L. Woodruff	A. F. Shull—5 years
1919. C. M. Child	H. H. Wilder	D. H. Tennent—5 years
1920. Gilman A. Drew	Caswell Grave	R. P. Bigelow—5 years
1921. Charles A. Kofoid	Aaron T. Treadwell	L. J. Cole—4 years
1922. H. H. Wilder	Bennet M. Allen	H. V. Wilson—5 years
1923. M. F. Guyer	Robert A. Budington	M. M. Metcalf—5 years
1924. R. G. Harrison	R. K. Nabours	George Lefevre—5 years
1925. C. R. Stockard	C. R. Moore	C. M. Child—5 years
1926. S. O. Mast	W. C. Allee	G. A. Drew—5 years
1927. S. J. Holmes	Robert Chambers, Jr.	C. A. Kofoid—5 years
1928. Caswell Grave	M. H. Jacobs	H. H. Wilder—5 years
1929. C. B. Davenport	Fernandus Payne	J. H. Gerould—1 year
1930. H. V. Neal	E. E. Just	M. F. Guyer—5 years
1931. Fernandus Payne	D. E. Minnich	R. G. Harrison—5 years
1932. W. C. Curtis	L. V. Heilbrunn	C. R. Stockard—5 years
1933. Charles Zeleny	J. H. Bodine	S. O. Mast—5 years
1934. A. H. Sturtevant	H. W. Rand	S. J. Holmes—5 years
1935. R. W. Hegner	Sewall Wright	Caswell Grave—5 years
1936. W. C. Allee	C. W. Metz	C. B. Davenport—4 years
		H. V. Neal—5 years
		Fernandus Payne—5 years

Secretary-Treasurer

1914-1918. Caswell Grave	1918-1921. W. C. Allee
<i>Secretary</i>	<i>Treasurer</i>
1921-1924. W. C. Allee	1922. D. H. Tennent
1925-1928. D. E. Minnich	1923-1924. R. H. Bowen
1928-1929. L. B. Arey	1925-1930. L. B. Arey
1929-1930. D. E. Minnich	1930-1933. A. B. Dawson
1930-1933. W. H. Cole	1933-1935. B. H. Willier
1933-1936. H. B. Goodrich	

*Representative in the Division of Biology and Agriculture,
National Research Council*

M. F. Guyer 1919-1921	H. S. Jennings 1922-1925	E. G. Conklin 1923-1926
William Patten 1921-1924	L. L. Woodruff 1925-1928	H. S. Jennings 1929-1931
J. T. Patterson 1924-1927	E. G. Conklin 1926-1929	L. C. Dunn 1931-1932
G. H. Parker 1926-1929	F. R. Lillie 1919-1923	J. H. Bodine 1932-1935
G. H. Parker 1919-1922		

Associate Editors of the Journal of Morphology

1921.	Gary N. Calkins	J. S. Kingsley	William Patten
1921-1922.	E. G. Conklin	M. F. Guyer	W. M. Wheeler
1921-1923.	C. A. Kofoed	F. R. Lillie	J. T. Patterson
1922-1924.	G. A. Drew	H. V. Neal	L. L. Woodruff
1923-1925.	Caswell Grave	D. H. Tennent	H. V. Wilson
1924-1926.	Helen Dean King	S. O. Mast	A. A. Schaeffer
1925-1927.	R. W. Hegner	Fernandus Payne	H. B. Ward
1926-1928.	L. J. Cole	J. H. Gerould	M. H. Jacobs
1927-1929.	W. C. Curtis	J. Percy Moore	A. F. Shull
1928-1930.	Edgar Allen	G. A. Baitsell	R. E. Coker
1929-1931.	S. R. Detwiler	R. S. Lillie	H. J. Muller
1930-1932.	W. R. Coe	E. N. Harvey	H. E. Jordan
1931-1933.	L. V. Heilbrunn	S. O. Mast	G. K. Noble
1932-1934.	D. E. Minnich	B. H. Willier	L. L. Woodruff
1933-1935.	L. R. Cleveland	F. L. Hisaw	Franz Schrader
1934-1936.	Charles Zeleny	J. F. Daniel	J. S. Nicholas

LIST OF PLACES OF MEETING

AMERICAN MORPHOLOGICAL SOCIETY

1890—Boston	1894—Baltimore	1899—New Haven
1891—Philadelphia	1895—Philadelphia	1900—Baltimore
1892—Princeton	1896—Boston	1901—Chicago
1893—New Haven	1897—Ithaca	1902—Washington
	1898—New York	

CENTRAL NATURALISTS

1899—Chicago	1900—Chicago
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SOCIETY OF AMERICAN ZOÖLOGISTS

1901—Chicago	1902—Washington
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AMERICAN SOCIETY OF ZOÖLOGISTS

EASTERN BRANCH	JOINT MEETINGS	CENTRAL BRANCH
1903—Philadelphia	1905—Ann Arbor	1903—St. Louis
1904—Philadelphia	1908—Baltimore	1905—(Mch.) Chicago
1906—New York	1911—Princeton	1907—(Mch.) Madison
1907—New Haven	1912—Cleveland	1907—Chicago
1909—Boston	1913—Philadelphia	1910—(Apr.) Iowa City
1910—Ithaca		1910—Minneapolis
		1912—(Apr.) Urbana

MEETING PLACES

1914—Philadelphia	1922—Boston	1930—Cleveland
1915—Columbus	1923—Cincinnati	1931—New Orleans
1916—New York	1924—Washington	1932—Atlantic City
1917—Minneapolis	1925—New Haven	1933—Boston
1918—Baltimore	1926—Philadelphia	1934—Pittsburgh
1919—St. Louis	1927—Nashville	1935—Princeton
1920—Chicago	1928—New York	1936—Atlantic City
1921—Toronto	1929—Des Moines	

PROGRAM FOR THE THIRTY-FIFTH ANNUAL
MEETING OF THE AMERICAN SOCIETY
OF ZOÖLOGISTS

INDIANAPOLIS, INDIANA

DECEMBER 28, 29, 30, 1937

The Society meets this year in conjunction with Section F of the American Association for the Advancement of Science and in association with other biological societies. All meetings of the Society for reading of papers, demonstrations and exhibits will be held at the Indiana University School of Medicine in Indianapolis.

As in recent years, several special sessions have been arranged, three of which will be held jointly with other societies. A symposium on 'Sex Differentiation,' organized by Dr. B. H. Willier will be held in conjunction with the Genetics Society of America. A joint session, arranged by Prof. Chancey Juday, will be held with the Limnological Society of America on the subject of 'Hydrobiology.' A special session on the 'Physical Study of Protoplasm,' arranged by Dr. L. V. Heilbrunn will be held with the Society of Cellular Physiologists. In addition two other special programs have been organized. Dr. Ethel Browne Harvey will lead a program on 'Nucleus and Cytoplasm' and Dr. J. S. Nicholas on the 'Essentials of Mammalian Development.'

The annual dinner for all zoölogists and friends will be held on Wednesday evening in the Riley Room of the Claypool Hotel. Dr. Ralph S. Lillie, vice-president of Section F of the A.A.A.S. will give an address on the 'Nature of Organizing Action.'

The attention of members of the Society is directed also to the symposium on the 'Nature of Protoplasm,' arranged by the American Society of Naturalists, which will be held on Thursday afternoon in the Assembly Room of the Claypool Hotel.

The demonstration session of the Society will be held on Wednesday afternoon. Any member of the Society who desires to present an exhibit at the general scientific exhibition of the A.A.A.S., but not to be included in the program of the Society of Zoölogists, should communicate at once with Dr. F. C. Brown, director of exhibits for the A.A.A.S., Smithsonian Institution Building, Washington, D. C.

The Secretary has received no notification of special reduction in railroad fares for the Indianapolis meeting. However, members are advised to consult their local railroad ticket agents in regard to possible convention or holiday rates.

As in former years the Society is greatly indebted to The Wistar Institute for prompt publication and mailing of this program.

LUNCHEON AND DINNER TICKETS

Arrangements have been made for Biologists' Luncheons on Tuesday, Wednesday and Thursday in the Nurses Dining Room of the Riley Hospital, which is about one block from the Medical School. Luncheon tickets can be obtained at the Information Desk in the Medical School. Tickets for the Zoölogists' Dinner are also on sale at the Information Desk. In order to assist the Local Committee in making arrangements, members of the Society are urged to obtain luncheon and dinner tickets as early as possible.

TRANSPORTATION IN INDIANAPOLIS

The Indiana University School of Medicine, where all scientific sessions of the Society will be held, is situated at 1040 West Michigan Street, about 1 mile from the Claypool Hotel. Both West Michigan Street and West Tenth Street trackless trolleys provide transportation to the Medical School. These cars can be obtained at the northeast corner of Illinois and Washington Streets (across from the Claypool Hotel), and they unload directly in front of the Medical School.

Taxicab companies will offer special rates between the Claypool Hotel and the Medical School. Inquiries should be made either at the hotel or at the information desk in the Medical School.

PROGRAM

The opening session of the American Association for the Advancement of Science will be held at 8.15 on Monday evening, December 27th in the Auditorium of the Murat Temple. An address will be given by the Retiring President of the Association, Dr. Edwin Grant Conklin of Princeton University, on 'Science and Ethics.'

TUESDAY, DECEMBER, 28TH

- 9.30 A.M. Section A. *Embryology*. Papers 1 to 12. Room 29, Medical School.
Section B. *Physiology*. Papers 13 to 25. Room 116, Medical School.
Section C. *Endocrinology*. Papers 26 to 38. Auditorium, Medical School.
Section D. Joint Session with the Society of Cellular Physiologists on the *Physical Study of Protoplasm*. Dr. L. V. Heilbrunn, presiding. Papers 39 to 43. Room 134, Medical School.
- 11.30 to 1.30 *Biologists' Luncheon*. Cafeteria, Nurses Dining Room, Riley Hospital.
- 2.00 P.M. Joint Symposium with the Genetics Society of America on *Sex Differentiation*. Auditorium, Medical School. Dr. B. H. Willier, presiding.
1. Aberrant forms in *Drosophila* and sex differentiation. J. T. Patterson, University of Texas.
 2. Hormonal control of sex differentiation. R. K. Burns, University of Rochester.
 3. The role of hormones in the differentiation of secondary sex characters. L. V. Domm, The University of Chicago.
 4. Intersexuality and Development. Richard B. Goldschmidt, University of California.
- 9.15 P.M. *Biologists' Smoker*, Claypool Hotel.

WEDNESDAY, DECEMBER 29TH

- 9.30 A.M. Section A. Special program on *The Essentials of Mammalian Development*. Dr. J. S. Nicholas, presiding. Papers 44 to 47. Auditorium, Medical School.
Section B. *Physiology and Ecology*. Papers 48 to 57. Room 29, Medical School.
Section C. *Endocrinology*. Papers 58 to 69. Room 134, Medical School.
Section D. *Protozoölogy and Cytology*. Papers 70 to 78. Room 116, Medical School.
- 12.00 M. Annual Business Meeting of Section F, A.A.A.S., Auditorium, Medical School.
- 12.10 P.M. Annual Business Meeting of the American Society of Zoölogists, Auditorium, Medical School.

- 11.30 to 1.30 *Biologists' Luncheon.* Cafeteria, Nurses Dining Room, Riley Hospital.
- 2.00 P.M. Special Program on *Nucleus and Cytoplasm.* Dr. Ethel Browne Harvey, presiding. Papers 79 to 82. Room 29, Medical School.
- 2.00 P.M. *Demonstrations.* Papers 126 to 155. Rooms 211, 35 and 39, Medical School. (Other rooms may be announced.)
- 7.00 P.M. *Zoölogists' Dinner.* Address by Dr. Ralph S. Lillie, vice-president of Section F, on *The Nature of Organizing Action.* Riley Room, Claypool Hotel. Open to all zoölogists and friends.

THURSDAY, DECEMBER 30TH

- 9.30 A.M. Section A. Joint Session with the Limnological Society of America on *Hydrobiology.* Professor Chancey Juday, presiding. Papers 83 to 88. Auditorium, Medical School.
- Section B. *Physiology.* Papers 89 to 104. Room 29, Medical School.
- Section C. *Cytology.* Papers 105 to 118. Room 134, Medical School.
- Section D. *General Morphology and Miscellaneous.* Papers 119 to 125. Room 116, Medical School.
- 11.30 to 1.30 *Biologists' Luncheon.* Cafeteria, Nurses Dining Room, Riley Hospital.
- 2.00 P.M. Joint Session with the American Society of Naturalists on *The Nature of Protoplasm.* Assembly Room, Claypool Hotel.
1. The reproduction of virus proteins. W. M. Stanley, Rockefeller Institute for Medical Research.
 2. The chemical nature of proteins as revealed by permeability. S. C. Brooks, University of California.
 3. The physical state of protoplasm in certain cells. Robert Chambers, New York University.

The Naturalists' Dinner will be held on Thursday at 6.30 P.M. in the Claypool Hotel. The presidential address will be given by Dr. D. H. Tennent, Bryn Mawr College, on "Some Problems in the Study of Photosensitization."

Attention is also directed to the demonstration program of the Genetics Society of America on Tuesday and Wednesday forenoons, and to the demonstration program of the American Society of Parasitologists on Wednesday afternoon. All demonstrations will be held in the laboratories of the Medical School.

LIST OF TITLES

TUESDAY MORNING SESSION, DECEMBER 28TH

This session will be held in four sections, as shown below

Section A. *Embryology*, 9.30 A.M.; Room 29, Medical School

1. The chick embryo as a test organism in the study of the mechanism of carbohydrate metabolism. (10 min.) (Lantern.) Albert J. Dalton and Ramon F. Hanzal, Western Reserve University.
2. The effect of humidity on the developmental rate of chick embryos incubated under increased atmospheric pressure. (10 min.) (Lantern.) Bert Cunningham, Duke University.
3. Differentiation capacity of pieces of the chick blastoderm in early primitive streak stages. (15 min.) (Lantern.) Dorothea Rudnick, University of Rochester.
4. Self-differentiation of the axes and of the skeleton in transplanted limb primordia of chick embryos. (10 min.) (Lantern.) Viktor Hamburger and Molly Waugh, Washington University, St. Louis.
5. Determination of the dorsoventral axis of the forelimb in *Amblystoma microstomum*. (10 min.) Harvey B. Lovell, University of Louisville. (Introduced by A. R. Middleton.)
6. The effect of dead optic vesicle upon explants of prospective ectoderm. (12 min.) (Lantern.) C. D. Van Cleave, University of Pennsylvania.
7. Experiments on the development of the tail and related structures in *Urodela*. (15 min.) (Lantern.) Paul L. Risley, State University of Iowa.
8. Effect of diet on the small intestine of *Rana sylvatica* larvae. (12 min.) (Lantern.) Ralph G. Janes, Wayne University College of Medicine.
9. Auxin yields from frog tissues and embryos (*Rana pipiens*). (15 min.) (Lantern.) True W. Robinson, University of Illinois. (Introduced by Leigh Hoadley.)
10. Analysis of the development of fish embryos by means of the mitotic index. V. The processes of early differentiation of organs in *Fundulus heteroclitus*. (15 min.) (Lantern.) Roy W. Jones, Central State Teachers College, Edmond, Okla. (Introduced by Aute Richards.)
11. Adaptations for viviparity in embryos and ovary of *Anableps anableps*. (10 min.) C. L. Turner, Northwestern University.
12. Certain aspects of swim bladder and stomach pouch development in *Hemichromis bimaculata*. (15 min.) (Lantern.) Robert S. McEwen, Oberlin College.

The program in embryology is continued Wednesday morning with paper no. 44.

Section B. *Physiology*, 9.30 A.M.; Room 116, Medical School

13. The unusual mortality that characterizes a *Paramecium* culture. (9 min.) (Lantern.) Edgar P. Jones, University of Akron.
14. The potential longevity of a *Paramecium* culture. (6 min.) (Lantern.) Edgar P. Jones, University of Akron.
15. The relation between the number of individuals per volume of culture solution and rate of growth in *Chilomonas paramecium*. (15 min.) (Lantern.) S. O. Mast and D. M. Pace, Johns Hopkins University.
16. The relation between hydrogen ion concentration of the culture medium and rate of growth in *Chilomonas paramecium*. (10 min.) (Lantern.) S. O. Mast and D. M. Pace, Johns Hopkins University.
17. The rate of pulsation and the function of the contractile vacuole in *Paramecium multimicronucleatum*. I. Relation between feeding and the rate of pulsation of the contractile vacuoles. (5 min.) (Lantern.) John A. Frisch, Johns Hopkins University and Canisius College.
18. The rate of pulsation and the function of the contractile vacuole in *Paramecium multimicronucleatum*. II. Relation between locomotion and the rate of pulsation of the contractile vacuoles. (10 min.) (Lantern.) John A. Frisch, Johns Hopkins University and Canisius College.
19. Quantitative determination of dextrose in cultures of *Colpidium*, *Glaucoma*, *Chilomonas* and *Chlorogonium*. (8 min.) (Lantern.) John B. Loefer, Berea College and New York University.
20. Compression studies on amebas. (12 min.) (Lantern.) A. A. Schaeffer, Temple University.
21. The relationship between the reactions of *Amoeba* to light and alternating current. (15 min.) (Lantern.) H. T. Folger, Harold Thomas, Fred Alsup, Fisk University.
22. Production of auxin by yeast (*Saccharomyces cerevisiae*). (15 min.) (Lantern.) True W. Robinson, University of Illinois. (Introduced by Charles Zeleny.)
23. Some effects of numbers present on rate of cytolysis of *Euplanaria dorotocephala* following ultra-violet radiation. (15 min.) (Lantern.) W. C. Allee and Janet Wilder, The University of Chicago.
24. Rate of growth of *Daphnia longispina* as affected by fish-conditioned water and by certain fish extracts. (15 min.) (Lantern.) Lester Ingle and Asher Finkel, The University of Chicago and the University of Illinois.
25. Some aspects of the behavior of the fresh water jellyfish, *Craspedacusta* sp. (12 min.) (Lantern.) Lorus J. Milne, Randolph-Macon Woman's College, Lynchburg, Va. (Introduced by J. I. Hamaker.)

The program in physiology is continued Wednesday morning with paper no. 48.

Section C. *Endocrinology*, 9.30 A.M.; Auditorium, Medical School

26. Effect of dihydroandrosterone and testosterone on the White Leghorn chick. (15 min.) (Lantern.) W. R. Breneman, Indiana University.
27. Some effects of progestin-containing extracts and progesterone on young male albino rats. (9 min.) (Lantern.) J. K. Lamar, The University of Chicago. (Introduced by Carl R. Moore.)

28. The gonadotropic and adrenotropic content of the pituitary gland of the chicken. (10 min.) (Lantern.) Roland K. Meyer, C. H. Mellish and Herbert Kupperman, University of Wisconsin.
29. Gonadotropic inhibitory substance and precipitins in the blood of monkeys receiving gonadotropic hormone preparations. (10 min.) (Lantern.) Harold R. Wolfe and Roland K. Meyer, University of Wisconsin.
30. Augmentation of pituitary gonadotropic extracts by heme. (15 min.) (Lantern.) L. E. Casida, Roland K. Meyer and W. H. McShan, University of Wisconsin. (Introduced by Leon J. Cole.)
31. The action of pregnancy urine hormone (PU) on the female genital tract of castrated monkeys. (15 min.) (Lantern.) Frederick L. Hisaw, R. O. Greep and H. L. Fevold, Harvard University.
32. Variation of gonad-stimulating material in the hypophysis of male ground squirrels. (15 min.) (Lantern.) L. J. Wells, University of Missouri.
33. Factors influencing the character of heat in the guinea pig. (15 min.) (Lantern.) William C. Young, Edward W. Dempsey and Carl W. Hagquist, Brown and Yale Universities.
34. The reticulo-endothelial system and hormone refractoriness. I. Effect of prolactin on the young normal and splenectomized male rat. (8 min.) (Lantern.) Albert S. Gordon, William Kleinberg and Harry A. Charipper, Washington Square College, New York University.
35. The reticulo-endothelial system and hormone refractoriness. II. Effect of thyrotropic hormone on the young normal, splenectomized, and 'dye-blocked' guinea pig. (7 min.) (Lantern.) Albert S. Gordon, William Kleinberg and Harry A. Charipper, Washington Square College, New York University.
36. Comparative effects of light stimulation and administration of gonadotropic hormones on female sparrows. (15 min.) (Lantern.) Gardner M. Riley and E. Witschi, State University of Iowa. (Introduced by Frank A. Stromsten.)
37. Endocrine amphisexuality in the female starling (*Sturnus vulgaris*). (5 min.) (Lantern.) Emil Witschi, Richard A. Miller and William Hart, State University of Iowa.
38. Prolongation of the life of the corpus luteum by hysterectomy in the rat. (15 min.) (Lantern.) James T. Bradbury, University of Michigan. (Introduced by G. R. La Rue.)

The program in endocrinology is continued Wednesday morning with paper no. 58.

Section D. *Joint Session with the Society of Cellular Physiologists*, 9.30 A.M.;
Room 134, Medical School

THE PHYSICAL STUDY OF PROTOPLASM

L. V. Heilbrunn, presiding

39. Optical studies on the structure of protoplasm. Francis O. Schmitt, Washington University.
40. Some problems on cellular physiology at sub-zero temperatures. Basile J. Luyet, St. Louis University.

41. Some effects of ultracentrifuging upon the physical structure of protoplasm. H. W. Beams, State University of Iowa.
42. The effect of thermal and electrical stimulation on the viscosity of ameba protoplasm. C. A. Angerer, University of Pennsylvania. (Introduced by the Secretary.)
43. The electric charge of protoplasmic colloids. L. V. Heilbrunn, University of Pennsylvania.

TUESDAY AFTERNOON SESSION, DECEMBER 28TH

2.00 P.M., Auditorium, Medical School

Joint Symposium with the Genetics Society of America

SEX DIFFERENTIATION

B. H. Willier, presiding

1. Aberrant forms in *Drosophila* and sex differentiation. J. T. Patterson, University of Texas.
2. Hormonal control of sex differentiation. R. K. Burns, University of Rochester.
3. The role of hormones in the differentiation of secondary sex characters. L. V. Domm, The University of Chicago.
4. Intersexuality and development. Richard B. Goldschmidt, University of California.

TUESDAY EVENING SESSION, DECEMBER 28TH

Biologists' Smoker, 9.15 P.M.; Claypool Hotel

WEDNESDAY MORNING SESSION, DECEMBER 29TH

This session will be held in four sections, as shown below

Section A. *Special Program in Embryology, 9.30 A.M.; Auditorium Medical School*

THE ESSENTIALS OF MAMMALIAN DEVELOPMENT

J. S. Nicholas, presiding

44. The ovulation process and the migration of the rat ovum. (Motion picture.) J. S. Nicholas and Edgar Allen, Osborn Zoölogical Laboratory and Department of Anatomy, Yale University.
45. Origin of the yolk sac in Primates. G. L. Streeter, Department of Embryology, Carnegie Institution of Washington, Baltimore.
46. The mammalian fetal membranes and placenta; their development, evolution and phylogenetic significance. H. W. Mossman, Department of Anatomy, University of Wisconsin.
47. The development of reflexes in the mammalian fetus. Davenport Hooker, University of Pittsburgh, School of Medicine.

Section B. *Physiology and Ecology*, 9.30 A.M.; Room 29, Medical School

48. Experimental studies of the factors controlling the establishment of transmissive nerve muscle connections: Occurrence of hyperneurotization. (15 min.) William Beggs Fort, The University of Chicago. (Introduced by Paul A. Weiss.)
- 48 A. Conduction of excitation in vertebrate smooth muscle. (12 min.) (Lantern.) Emil Bozler, Ohio State University. (Introduced by Hans O. Haterius.)
49. Rods and cones in the retina of *Fundulus heteroclitus*, and the regions of the retina related to the different chromatophoric responses. (15 min.) (Lantern.) Earl O. Butcher, Hamilton College.
50. The control of the color changes in the dogfish, *Mustelus* by neurohumors. (15 min.) (Lantern.) G. H. Parker, Harvard University.
51. The relationship of the pars tuberalis to melanophore response in frogs (*Rana pipiens*). (6 min.) (Lantern.) A. L. Soderwall and F. R. Steggerda, Departments of Zoölogy and Physiology, University of Illinois.
52. The light response of *Clitellio arenarius* (O. F. Müller). (15 min.) (Moving pictures, Lantern.) D. E. Minnich, University of Minnesota.
53. Effect of lesions of the corpus striatum on the brooding behavior of cichlid fishes. (15 min.) (Lantern.) G. K. Noble, American Museum of Natural History.
54. Movement of stream fish. (15 min.) R. V. Bangham and N. L. Bennington, College of Wooster and Beloit College.
55. The asphyxial oxygen concentration for three species of fish. (10 min.) (Lantern.) James L. Wilding, Indiana University. (Introduced by Fernandus Payne.)
56. The history of a pelagic population. (15 min.) (Lantern.) Alfred C. Redfield, Harvard University.
57. Fauna of Yucatan caves. (15 min.) (Lantern.) A. S. Pearse, Duke University.

The program in physiology is continued Thursday morning with paper no. 89.

Section C. *Endocrinology*, 9.30 A.M.; Room 134, Medical School

58. Effects of salivary gland ablation on young albino rats. (6 min.) (Lantern.) J. C. Plagge, The University of Chicago. (Introduced by Carl R. Moore.)
59. Rat prostate and seminal vesicle grafts in relation to the sex and sex-hormone state of the hosts. (15 min.) (Lantern.) Dorothy Price, The University of Chicago.
60. A specific action of the anterior pituitary on the intestine. (10 min.) (Lantern.) J. P. Schooley, O. Riddle and R. W. Bates. Carnegie Institution, Department of Genetics.
61. The effect of ovarian hormones and seasons on the thyroids of both sexes of *Anolis carolinensis*. (15 min.) (Lantern.) L. T. Evans, Montana State University.
62. Studies on the physiology of avian thyroid. (10 min.) (Lantern.) Dorothea Starbuck Miller, State University of Iowa. (Introduced by E. Witschi.)
63. Testicular function in hyperthyroidism. (15 min.) (Lantern.) George K. Smelser, Columbia University.

64. A blood sugar increasing effect of parathyroid extracts. (5 min.) (Lantern.) Oscar Riddle and Louis B. Dotti. Carnegie Institution, Department of Genetics.
65. The endocrine basis for the rise in blood calcium associated with egg laying in the fowl. (15 min.) (Lantern.) Margaret Altmann and F. B. Hutt, Cornell University.
66. The effectiveness of theelin in normal and castrated adrenalectomized female rats. (15 min.) (Lantern.) Robert L. Kroc, Indiana University.
67. The separability of hypophyseal lactogenic and corticotropic activities. (12 min.) (Lantern.) Warren O. Nelson and Charles E. Tobin, Wayne and Yale Universities.
68. Post-adrenalectomy diuresis and related considerations. (15 min.) (Lantern.) Robert Gaunt, Hugh E. Potts and Eleanor Loomis, Washington Square College, New York University.
69. The effect of the reduction of adrenal tissue on the response of the albino rat to cold. (10 min.) (Lantern.) S. M. Horvath, F. A. Hitchcock and F. A. Hartman, Ohio State University.

Section D. *Protozoölogy and Cytology*, 9.30 A.M.; Room 116, Medical School

70. Studies on living conjugants of *Paramecium caudatum*. (15 min.) (Motion picture.) Ralph Wichterman, Temple University.
71. Nuclear division in *Chaos chaos* Linnaeus. (15 min.) (Lantern.) M. Catherine Hinchey, Temple University. (Introduced by D. H. Wenrich.)
72. Cannibalism and gigantism among the protozoa. (15 min.) (Lantern.) A. C. Giese, Stanford University. (Introduced by C. V. Taylor.)
73. The organization of the parabasal apparatus of certain polymastigote flagellates. (15 min.) (Lantern.) Harold Kirby, Jr., University of California.
74. Osmiophilic bodies in the ciliate, *Tillina canalifera*. (10 min.) (Lantern.) John P. Turner, University of Minnesota.
75. Intracellular parasitism in fish producing a gigantic growth of the infected cells. (15 min.) (Lantern.) Richard Weissenberg, Washington University, St. Louis. (Introduced by Roscoe R. Hyde.)
76. Growth of the intracellular symbionts of the cockroach, *Periplaneta americana*. (10 min.) (Lantern.) H. T. Gier, Harvard University and Ohio University.
77. A study of the ciliated epithelium of the gill of *Nucula castrensis*, Hinds. (10 min.) S. I. Kornhauser and Dorothy Blumer, Department of Anatomy, University of Louisville.
78. Some variations in certain stages of living germ cells of insects. (15 min.) (Motion picture.) W. J. Baumgartner, University of Kansas.

The program in Cytology is continued Thursday morning with paper no. 105.

Business Meeting

The *business meeting of Section F, A.A.A.S.* will be held on December 29th, 12.00 M.; Auditorium, Medical School. The *business meeting of the American Society of Zoölogists* will be held at 12.10 P.M. immediately after the meeting of Section F.

WEDNESDAY AFTERNOON SESSION, DECEMBER 29TH

Special Program, 2.00 P.M.; Room 29, Medical School

NUCLEUS AND CYTOPLASM

Ethel Browne Harvey, presiding

- 79. Determination of polarity in the Fucus egg by centrifuging. D. M. Whitaker, Stanford University.
- 80. Development of eggs without nuclei or chromosomes. Ethel Browne Harvey, Princeton University.
- 81. Interfacial phenomena between oil drops and cytoplasm. M. J. Kopac, Washington Square College, New York University.
- 82. Colloidal conditions of the nucleus. (Motion picture.) William R. Duryee, Washington Square College, New York University.

DEMONSTRATIONS

Beginning at 2.00 P.M.; Rooms 211, 35 and 39, Medical School

(Other rooms may be announced)

Motion picture demonstrations

- 126. Reproduction among mammals. Herluf H. Strandskov, The University of Chicago. Motion picture to be shown at 3.00 P.M.; Room 35.
- 127. Ovulation and egg formation in the hen. D. C. Warren and R. E. Phillips, Kansas State College. Motion picture to be shown at 3.15 P.M.; Room 35.
- 128. The 'resonance' character of nervous control, demonstrated by the 'homologous' response of transplanted muscles and limbs. Paul Weiss, The University of Chicago. Motion picture to be shown at 3.45 P.M.; Room 35.

Embryology

- 129. Transplanted wings and hind limbs of chick embryos prepared by the methylene blue method. Molly Waugh and Viktor Hamburger, Washington University, St. Louis.
- 130. The hormones of the hypophysis of the turkey. G. M. Riley, A. J. Stanley and E. Witschi, State University of Iowa.
- 131. Abnormalities induced by centrifuging frog eggs. H. W. Beams and R. L. King, State University of Iowa.
- 132. The effect of pituitary implantation on advancing sexual maturity in Ameiurus melas. Waldo Shumway and Arnold L. Soderwall, Department of Zoölogy, University of Illinois.
- 133. Adaptations for viviparity in embryos and ovary of Anableps anableps. C. L. Turner, Northwestern University.
- 134. Axial duplication in serpents. Bert Cunningham, Duke University.
- 135. Some mammalian abnormalities. Albert M. Reese, West Virginia University.

Cytology and Protozoölogy

136. The structure of salivary chromosomes in *Simulium*. T. S. Painter and Allen B. Griffen, University of Texas.
137. An experimental study of chromatin diminution in *Ascaris*. R. L. King and H. W. Beams, State University of Iowa.
138. Demonstrations of X-Y sex chromosomes in first spermatocytes of man. R. L. King and H. W. Beams, State University of Iowa.
139. Multiple chromosomes of *Paratylotropidia brunneri* (Orthoptera, Acrididae). R. L. King and H. W. Beams, State University of Iowa.
- 139 A. Nuclear division in *Chaos chaos* Linnaeus. M. Catherine Hinchey, Temple University. (Introduced by D. H. Wenrich.)
140. Sperm production in rams in relation to the thyroids and pituitaries. Victor Berliner and Virgene Warbritton, Department of Animal Husbandry of the Agricultural Experiment Station of the University of Missouri and the United States Department of Agriculture, cooperating.
141. Viscosities of tumor and of normal cells as determined by the ultra-centrifuge. M. F. Guyer and P. E. Claus, University of Wisconsin.
142. The vacuoles and vacuolar activities of the marine *Amoeba*, *Flabellula mira*. D. L. Hopkins, Johns Hopkins University.
143. The unusual mortality that characterized a *Paramecium* culture. Edgar P. Jones, University of Akron.
144. Intracellular parasitism in fish producing a gigantic growth of the infected cells. Richard Weissenberg, Washington University, St. Louis. (Introduced by Roscoe B. Hyde.)
145. Several substitutes for the standard microtome knife. Robert T. Hance, University of Pittsburgh.
146. A microtome that students can make. Robert T. Hance. University of Pittsburgh.

Physiology and Endocrinology

147. A mechanical demonstration of the effects and interrelationships of the human female hormones of reproduction. Wayne L. Whitaker and R. Dean Schick, University of Michigan. (Introduced by Alvalyn E. Woodward.)
148. Experimental production of exophthalmos comparable to that found clinically. George K. Smelser, Columbia University.
149. Effects of theelin upon the reproductive system of male *Anolis carolinensis*. M. L. Clapp, Montana State University. (Introduced by L. T. Evans.)
150. Sex reversal in the fighting fish, *Betta splendens*. G. K. Noble and K. F. Kumpf, American Museum of Natural History.
151. The hormonal function of the corpora allata in the grasshopper, *Melanoplus differentialis*. Isabelle G. Weed, State University of Iowa. (Introduced by H. W. Beams.)
152. A new method for simultaneously recording the electrical and mechanical changes in the insect heart. Frederick Crescitelli and Theodore L. Jahn, State University of Iowa.
153. A new kymographic ink-recording pen for the physiological laboratory. T. L. Patterson, Wayne University.

Miscellaneous

154. Evolution of a gastropod species. H. B. Stenzel and R. W. Cumley, University of Texas.
155. The snakes of the United States and Canada. A. H. Wright and A. A. Wright, Cornell University.

WEDNESDAY, DECEMBER 29TH, 7.00 P.M.

Zoölogists' Dinner, Riley Room, Claypool Hotel

Address by Dr. Ralph S. Lillie, vice-president of Section F, on *The Nature of Organizing Action*.

(Open to all zoölogists and friends)

THURSDAY MORNING SESSION, DECEMBER 30TH

This session will be held in four sections, as shown below

Section A. *Joint meeting with the Limnological Society of America*, 9.30 A.M.;

Auditorium, Medical School

Chancey Juday, presiding

Symposium: HYDROBIOLOGY

83. The correlation of sea water temperatures and solar radiation with the phytoplankton calendar at Friday Harbor, Washington. Lyman D. Phifer, Oceanographic Laboratories, University of Washington. (Introduced by J. E. Guberlet.)
84. Photosynthesis in relation to light penetration into lake waters. W. M. Manning, Limnological Laboratory, University of Wisconsin. (Introduced by C. Juday.)
85. The relation between diatoms and copepods as a factor in the productivity of the sea. George L. Clarke, Harvard University and Woods Hole Oceanographic Institution.
86. The present status of work on the ecology of aquatic insects as shown by the work on the Odonata. Clarence H. Kennedy, Ohio State University and Franz Theodore Stone Laboratory. (Introduced by Raymond C. Osborn.)
87. Studies of the water flow through certain pelecypod molluscs and some applications thereof. (Motion picture.) T. C. Nelson, Rutgers University.
88. Contributions of limnology to game fish production. William J. K. Harkness, University of Toronto. (Introduced by A. G. Huntsman.)

Section B. *Physiology*, 9.30 A.M.; Room 29, Medical School

89. A new method of studying the motor activity of the stomach in a fish (*Scorpaenichthys marmoratus*). (15 min.) (Lantern.) T. L. Patterson, Wayne University and Hopkins Marine Station of Stanford University.
90. Sleep; biological significance and mechanism. (10 min.) F. H. Pike, Columbia University.

91. Physiological studies on hibernation in the chipmunk. (15 min.) (Lantern.)
Alvalyn E. Woodward and John M. Condrin, University of Michigan.
92. Renal excretion at low urine volumes. The calculation of 'minimal' urea clearances, and the mechanism of oliguria. (15 min.) (Lantern.) Leon
C. Chesley, Margaret Hague Maternity Hospital.
93. The effects of pituitrin on the water metabolism in *Ambystoma* during metamorphosis. (6 min.) (Lantern.) F. R. Steggerda, Department of
Physiology, University of Illinois.
94. Adjustments of body water contents in frogs. (10 min.) (Lantern.) E. F.
Adolph, University of Rochester.
95. Experimental analysis of the vasomotor reversal. (15 min.) (Lantern.)
Theodore Koppanyi, Charles R. Linegar and Robert P. Herwick, George-
town University, School of Medicine.
96. A comparison between the beat in three and four chambered hearts with
reference to embryological development. (6 min.) (Lantern.) Stephen
W. Gray, University of Illinois. (Introduced by F. R. Steggerda.)
97. Comparison of quantitative precipitin technics as influenced by injection
procedures. (10 min.) (Lantern.) Joseph G. Baier, Jr., and Harold R.
Wolfe, University of Wisconsin.
98. Differences in the permeability of the erythrocytes of two closely related
species. (12 min.) (Lantern.) M. H. Jacobs and H. N. Glassman,
University of Pennsylvania and A. K. Parpart, Princeton University.
99. Colchicine treatments with *Cladocera*. (15 min.) (Lantern.) H. Howard
Dunham, W. Malcolm Reid and A. M. Banta, Brown University and
Carnegie Institution of Washington.
100. Oxygen consumption as a function of pH in *Thyone*. (5 min.) (Lantern.)
Wm. A. Hiestand, Purdue University. (Introduced by R. M. Cable.)
101. Ultraviolet radiation of grasshopper embryos (*Melanoplus differentialis*).
(8 min.) (Lantern.) Malcolm Ray, State University of Iowa. (Introduced
by J. H. Bodine.)
102. Activation of naturally occurring enzymes. (7 min.) (Lantern.) J. H.
Bodine, T. H. Allen, E. J. Boell, State University of Iowa.
103. The hormonal function of the corpora allata in the grasshopper, *Melanoplus
differentialis*. (12 min.) (Lantern.) Isabelle G. Weed, State University
of Iowa. (Introduced by H. W. Beams.)
104. The growth and development of the female bee (*Apis mellifica* L.). (12 min.)
(Lantern.) R. M. Melampy, E. R. Willis and S. E. McGregor, Bureau of
Entomology and Plant Quarantine and the Louisiana State Agricultural
Experiment Station. (Introduced by Wm. H. Gates.)

Section C. *Cytology*, 9.30 A.M.; Room 134, Medical School

105. An experimental study of chromatin diminution in *Ascaris*. (10 min.)
(Lantern.) R. L. King and H. W. Beams, State University of Iowa.
106. The structure of salivary chromosomes in *Simulium*. (15 min.) (Lantern.)
T. S. Painter and Allen B. Griffen, University of Texas.
107. A study of the chromosomes of a sex reversed female and of normal males
of the common fowl, *Gallus domesticus*. (15 min.) (Lantern.) Richard
A. Miller, State University of Iowa. (Introduced by C. B. Davenport.)

108. Oogenesis of *Campeloma rufum*, a parthenogenetic snail. (10 min.) Norman T. Mattox, Purdue University. (Introduced by Harley J. Van Cleave.)
109. A cytological study of the ovaries of the bats, *Myotis lucifugus lucifugus* and *M. grisescens*. (12 min.) (Lantern.) Mary J. Guthrie and Katharine R. Jeffers, University of Missouri.
110. Further observations on the reproductive system in male bats. (8 min.) (Lantern.) Roland E. Miller, University of Missouri. (Introduced by Mary J. Guthrie.)
111. The Golgi substance of insect muscle. (12 min.) (Lantern.) Herbert L. Eastlick, University of Missouri. (Introduced by W. C. Curtis.)
112. On the ultrastructure of the batonettes of Heidenhain in renal tubule cells of amphibia as revealed by polarized light studies. (10 min.) (Lantern.) Caswell Grave II, Washington University, St. Louis. (Introduced by Francis O. Schmitt.)
113. The numerical relation of retinal ganglion cells to optic nerve fibers in vertebrates. (15 min.) (Lantern.) Leslie B. Arey, Northwestern University Medical School.
114. Observations on giant cells appearing in tissue cultures of amphibian spleen. (15 min.) (Lantern.) George A. Baitzell, Yale University.
115. Mitotic activity of stimulated rat adrenals and spleen measured by colchicine technique. (15 min.) (Lantern.) Opal Wolf, Goucher College.
116. The cellular changes induced in the testes of the albino rat by artificial cryptorchidism aided by the arrest of mitosis with colchicine. (15 min.) (Lantern.) Arthur J. Gatz, Carleton College. (Introduced by J. E. Wodsedalek.)
117. The stimulation of yeast growth by colchicine. (10 min.) (Lantern.) Oscar W. Richards, Research Section, Spencer Lens Co.
118. Ram sperm in the genital tract of the ewe. (10 min.) (Lantern.) Virgene Warbritton, Frederick N. Andrews, Victor Berliner and Fred F. McKenzie, Agriculture Experiment Station, University of Missouri and United States Department of Agriculture, cooperating.

Section D. *General Morphology and Miscellaneous*, 9.30 A.M.; Room 116,
Medical School

119. The blood vascular system of the hypophysis of *Amblystoma tigrinum*. (10 min.) (Lantern.) Paul G. Roofe, University of Louisville. (Introduced by Dr. S. I. Kornhauser.)
120. The development of the vallate papilla and associated taste buds in the rat. (10 min.) Theodore W. Torrey, Indiana University.
121. On the significance of the occurrence of melanin pigment in the corium of the skin or in the deeper portions of epidermal structures. (15 min.) (Lantern.) R. M. Strong, Loyola University, School of Medicine.
122. A comparison of organ-gland relationships in the arctic and the Ohio meadow mouse (*Microtus drummondi* and *Microtus pennsylvanicus*). (12 min.) (Lantern.) Daniel P. Quiring, Western Reserve University, Cleveland Clinic Foundation. (Introduced by J. Paul Visscher.)
123. Experimental study of survival value of acridian protective coloration. (15 min.) (Lantern.) F. B. Isely, Trinity University, Waxahachie, Texas.

124. The life-cycle of the hen louse *Lipeurus caponis* (Linn.). (10 min.) F. H. Wilson, Tulane University.
125. A method of determining the mean speed of movement of *Tribolium* beetles moving through a mass of flour. (10 min.) John Stanley, Queens University, Kingston, Ontario. (Introduced by Charles P. Sigerfoos.)

PAPERS PRESENTED BY TITLE

Embryology

156. Cumulative growth and time. Otto Glaser, Amherst College.
157. The numerical value of the heterogonic constant. Otto Glaser, Amherst College.
158. The geometrical basis for heterogonic correlation. Otto Glaser and Geo. P. Child, Amherst College.
159. Chemical nature of amphibian organizer. III. Chemical stimulation of the presumptive epidermis. L. G. Barth, Columbia University.
160. Fore-limb opercular perforation in *R. temporaria* and *R. bufo*. O. M. Helff, New York University.
161. The relation of the dorsal nerve cord and notochord to tail regeneration in anuran larvae. O. M. Helff, New York University.
162. The induction of pigment spots in *Styela partita*. S. Meryl Rose, Columbia University. (Introduced by Lester G. Barth.)
163. Induction of developmental acceleration by depressants. J. Wm. Buchanan, Northwestern University.
164. Observations of the early development of the zebra fish, *Brachydanio rerio*. Eduard Roosen-Runge, Brown University. (Introduced by J. W. Wilson.)
165. A preliminary study of sex development in turtle embryos following administration of testosterone. Paul L. Risley, State University of Iowa.
166. Histological evidence indicative of the natural occurrence of vitamin E deficiency in chick embryos. F. B. Adamstone, University of Illinois.
167. The response of the wings of *Depillate/wild*, *Depillate/vestigial*, and *Depillate/pennant* of *Drosophila melanogaster* to temperature. Morris H. Harnly, Washington Square College, New York University.
168. The histological study of the development of the compound eye in *Drosophila melanogaster*. Sibyl A. Hausman, Connecticut College. (Introduced by Dr. Pauline H. Dederer.)
169. The changes in the central nervous system during the life history of the beetle, *Passalus cornutus Fabricius*. I. E. Gray and Frances Perle Cody, Duke University.
170. The ossification of the chick skeleton. II. The skull. Carl J. Sandstrom and Herbert H. Pomerance, University College, New York University.
171. The ossification of the chick skeleton. III. The sternum. Carl J. Sandstrom and David F. Sunkin. University College, New York University.
172. Measurement of the specific conductance of the extra-embryonic fluids of the chick. Paul A. Walker, Connecticut State College.
173. The effect of ovum age at time of fertilization upon development during the pre-implantation period in the guinea pig. Richard J. Blandau and William C. Young, Brown and Yale Universities.

174. Homotransplantation of the embryonic mammalian digestive tract. A. J. Waterman, Williams College.
175. Preovulatory maturation of the rabbit's egg. A. J. Waterman and B. B. Owen, Williams College.
176. The early cleavage of *Polychoerus carmelensis*. Donald P. Costello, University of North Carolina.
177. Primary sexual phases in the oviparous oyster, *Ostrea virginica*. W. R. Coe, Yale University.

Physiology

178. Coalescence between coated and fresh oil drops. M. J. Kopac, Washington Square College, New York University.
179. The rate of protoplasmic streaming in *Elodea* cells at high hydrostatic pressure. Douglas Marsland, Washington Square College, New York University.
180. The viscosimeter method for determination of cell concentration in suspensions of living cells. Herbert Shapiro, Princeton University.
181. Enzyme activities in relation to fertilization. William Doyle, Bryn Mawr College.
182. The respiratory metabolism of blue crab nerves (*Callinectes sapidus*). Verlus F. Lindeman, Syracuse University.
183. A comparative study of the adductor muscle of the crayfish and sartorius muscle of the frog; with special reference to the action of electrolytes in response to electrical stimulation. Josephine Lamb Fernandes, Syracuse University. (Introduced by V. F. Lindeman.)
184. The action of acetylcholine and of inhibitory nerves upon the heart of *Venus*. C. Ladd Prosser and Hazel B. Prosser, Marine Biological Laboratory and Clark University.
185. The action of acetylcholine on the skeletal muscle fibers of the frog. Elsa M. Keil and F. J. M. Sichel, New Jersey College for Women and College of Medicine, University of Vermont.
186. A crustacean neurohumor. John H. Welsh, Harvard University.
187. Spectral composition of the light emitted by Jamaican fireflies. John Bonner Buck, Johns Hopkins University.
188. Studies in population physiology. VIII. The effect of larval population density on the post-embryonic development of the flour beetle, *Tribolium confusum* Duval. Thomas Park, The University of Chicago.
189. A note on the size and composition of old *Tribolium confusum* populations. Thomas Park, The University of Chicago.
190. Growth and survival of Japanese beetle larvae reared in different media. Daniel Ludwig and Henry Fox, University College, New York University.
191. The cause of the red-green color change in *Euglena rubra*. Leland P. Johnson and Theodore L. Jahn, Drake University, State University of Iowa, and the Iowa Lakeside Laboratory.
192. A study of the capacity-intensity factors in cyanide inhibition. Wilbur Robbie, State University of Iowa. (Introduced by J. H. Bodine.)
193. The responses of two species of opossums to selected stimuli. Elbert C. Cole, Williams College.

194. Concerning the effect on honeybees of lead arsenate, calcium arsenate, and phenothiazine as stomach poisons. Lloyd M. Bertholf, Western Maryland College and Bureau of Entomology and Plant Quarantine, United States Department of Agriculture.

Endocrinology

195. Specificity of the pituitary gonadotropic factors within the amphibia. Charles W. Creaser and Margaret Sheolnek, Wayne University, Detroit.
196. A comparative study of the normal relationship between pituitary and testis in three varieties of fowl. W. R. Breneman, Indiana University.
197. The effect of estrogens on the fish gonad. Philip Berkowitz, Washington Square College, New York University. (Introduced by Robert Gaunt.)
198. A quantitative study of experimentally induced heat in the spayed guinea pig. Vincent J. Collins, John L. Boling and William C. Young, Brown and Yale Universities.
199. The vaginal smear picture accompanying the oestrin-progesterone production of heat in the spayed guinea pig. John L. Boling, Vincent J. Collins and William C. Young, Brown and Yale Universities.
200. The relation of deciduomata to the reproductive cycle in the guinea pig. Edward W. Dempsey, Brown University and Harvard Medical School. (Introduced by A. D. Mead.)
201. Effects of testis removal on the fibro-muscular scrotal sac in the albino rat. L. J. Wells and M. D. Overholser, University of Missouri.
202. Cyclic changes in the thyroid and adrenal cortex of the male starling, *Sturnus vulgaris*, and their relation to the sexual cycle. J. Wendell Burger, Trinity College. (Introduced by T. H. Bissonnette.)
203. Sexual conditions in *Triturus viridescens*. Artificial hermaphroditism. (Female hosts with testicular grafts.) A. Elizabeth Adams, Mount Holyoke College.
204. Tests of the potency of thyroid and pituitary glands of tadpoles of *Rana clamitans*. A. Elizabeth Adams and Olive Bartholomew, Mount Holyoke College.
205. Hormones and sex determination in fishes and in frogs. E. Witschi and E. N. Crown, State University of Iowa.
206. Studies on the endocrines of teleosts. I. The morphology of the hypophysis of the goldfish (*Carassius auratus*). W. Randal Bell, Washington Square College, New York University. (Introduced by Harry A. Charipper.)
207. The efficacy of extracts from the bodies of functioning supplementary reproductives of termites in inhibiting or retarding neotenic sexual development in isolated nymphs. S. F. Light, Olga Hartman and O. H. Emerson, University of California, Berkeley.
208. Experimental production of exophthalmos in *Fundulus*. A. A. Abramowitz and H. L. Fevold, Harvard University.
209. On the specificity and related properties of the crustacean chromatophoretropic hormone. A. A. Abramowitz and R. K. Abramowitz, Marine Biological Laboratory, Woods Hole.

210. On the nature and the effects of a hormone-like substance produced on the spermatozoa of the oyster. T. C. Nelson and J. B. Allison, Rutgers University.
211. Normal and lasting sexual activity due to brephoplastic grafts of the hypophysis in hypophysectomized female rats. Raoul M. May, University of Paris.
212. Variations in chick testes. Frederick N. Andrews and Virgene Warbritton, Agriculture Experiment Station, University of Missouri and United States Department of Agriculture, cooperating.

Protozoölogy

213. The effect of tetanizing shocks on *Paramecium* and *Uroleptus*, with reference to injury and recovery. I. A. Tittler, Brooklyn College. (Introduced by B. R. Coonfield.)
214. (Paper withdrawn.)
215. The rate of pulsation and the function of the contractile vacuole in *Paramecium multimicronucleatum*. III. Relation between feeding and the relative rate of pulsation of the anterior and posterior contractile vacuoles. John A. Frisch, Johns Hopkins University and Canisius College.
216. *Phacus quinquemarginata*, sp. nov. Theodore L. Jahn and Fae M. Shawhan, State University of Iowa, Drake University and the Iowa Lakeside Laboratory.
217. The question of autotrophic nutrition in the plant-like flagellates, *Chlorogonium* and *Euglena*. R. P. Hall and H. W. Schoenborn, New York University.
218. Effects of different concentrations of sodium acetate on growth of *Euglena stellata*. R. P. Hall, New York University.
219. Vitamin B₁ and growth of protozoa. Alfred M. Elliott, Minnesota State Teachers College, Bemidji.
220. Plant hormones and growth of *Euglena* in relation to light. Alfred M. Elliott, Minnesota State Teachers College, Bemidji.
221. The incidence of protozoan parasites of frogs of the Okoboji region. William R. Bradley, State University of Iowa and the Iowa Lakeside Laboratory. (Introduced by H. W. Beams.)

Cytology

222. Chromosome number in larvae and neural-fold embryos of *Rana fusca* developed from pricked eggs with known polar body and early cleavage history. Charles L. Parmenter, University of Pennsylvania.
223. Mono-di-tri- and tetra-nucleate blood cells in parthenogenetically developed *Rana fusca* tadpoles. Charles L. Parmenter, University of Pennsylvania.
224. The occurrence and distribution of a third type of granular cell in the anterior pituitary of the cat. Alden B. Dawson and Harry B. Friedgood, Harvard University and Harvard Medical School.
225. The rate of mitotic activity in the pituitary of the growing rat. Gerard Roland Pomerat, Harvard University. (Introduced by Alden B. Dawson.)

226. Cytological study of chick heart muscle in tissue cultures. E. Frances Stilwell, Bryn Mawr College. (Introduced by D. H. Tennent.)
227. The Golgi apparatus in the proximal and distal tubule cells of the perfused frog's kidney. Victor M. Emmel, Brown University. (Introduced by J. Walter Wilson.)
228. Abnormalities induced by centrifuging frog eggs. H. W. Beams and R. L. King, State University of Iowa.

Morphology

229. The absorption of colloidal carbon from the body cavity of *Ammocoetes*. Theodore W. Torrey, Indiana University.
230. The ciliary ganglion and associated structures in the gecko, *Gymnodactylus kotschy*. L. T. Evans and J. Minckler, Montana State University.
231. The relation between eyeball size (retinal area) and the number of nerve fibers in the optic nerve of the dog. Leslie B. Arey, Northwestern University Medical School.
232. Cuticular scales and medulla of hairs from the Sei whale (*Balaenoptera borealis*). Leon A. Hausman, New Jersey College for Women. (Rutgers University.)
233. Weights and linear dimensions of the skull and of some of the long bones of the red tailed hawk (*Buteo borealis borealis*). Homer B. Latimer, University of Kansas.
234. External morphology of the whale shark, *Rhineodon typus*. L. Hussakof, American Museum of Natural History.
235. A comparison of the nervous system in normal and sinistral snails of the species *Campeloma rufum*. Alice Savage, University of Illinois. (Introduced by Harley J. Van Cleave.)

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236. Regeneration of the gonad tubules in *Thyone briareus* Lesueur following extirpation. Frank R. Kille, Swarthmore College.
237. Factors controlling regeneration in *Tubularia*. L. G. Barth, Columbia University.
238. Production of supernumerary outgrowths in planarians by the application of ethyl alcohol, aniline oil, and coal tar. El. D. Goldsmith, College of the City of New York and Washington Square College, New York University.
239. Anterior regeneration in the absence of central nervous tissues in the earthworm, *Eisenia foetida*. P. L. Bailey, Jr., College of the City of New York.

Ecology

240. Altitudinal distribution of fireflies in Jamaica. John Bonner Buck, Johns Hopkins University.
241. Light penetration in the Okoboji Lakes, summer 1937. Theodore L. Jahn and A. B. Taylor, State University of Iowa, University of Illinois and the Iowa Lakeside Laboratory.

General Evolution

242. A point in the relation between Charles Darwin and Thomas Huxley. Wm. E. Ritter, University of California.
243. The greatest biological problem. Alan Boyden, Rutgers University.
244. Termite nests—a study of the phylogeny of behavior. Alfred E. Emerson, The University of Chicago.

Miscellaneous

245. Anyphaenidae and Clubionidae of Michigan. A. M. Chickering, Albion College.
246. Distribution of mammals on Lake Michigan islands. Robert T. Hatt, Cranbrook Institute of Science.
247. Copulation in the acoelous turbellarian *Polychoerus carmelensis*. Donald P. Costello and Helen M. Costello, University of North Carolina.
248. Egg laying in the acoelous turbellarian *Polychoerus carmelensis*. Donald P. Costello and Helen M. Costello, University of North Carolina.
249. A trematode of genus *Maritrema* (Nicoll) parasitic in the barnacle and the ruddy turnstone. Charles E. Hadley and Ruth M. Castle, Montclair State Teachers' College and Vassar College.
250. The food of pickerel. George W. Hunter, III, Wesleyan University, and J. Stewart Rankin, Amherst College.

ABSTRACTS

An author index of abstracts begins on page 141.

PAPERS READ

1. THE CHICK EMBRYO AS A TEST ORGANISM IN THE STUDY OF THE MECHANISM OF CARBOHYDRATE METABOLISM. Albert J. Dalton and Ramon F. Hanzal, Western Reserve University. (Lantern; 10 min.)

The appearance of glycogen in the liver previous to the time when hormonal activity may be demonstrated makes the chick a valuable aid to the study of hormone action as related to carbohydrate metabolism.

Best's carmine stain was used for glycogen and Folin's micro-method was used to determine the blood sugar volume.

Tests so far made indicate that the hepatic cells of the 7- and 8-day chick embryo react readily to small amounts of thyroxin, insulin and eschatin (Parke, Davis and Co.) injected under the chorio-allantois. Thyroxin, 1/800 mg., injected on the fifth day causes the liver glycogen to disappear completely by the eighth day. Liver glycogen is rapidly reduced in amount after the injection of 0.1 cc. eschatin, an increase in the blood sugar concentration accompanying this reduction. Concentrations of 1/50,000 adrenalin produce no detectable change in the glycogen content. During the 20-hour period following the injection of 4 units insulin, an increase in liver glycogen occurs accompanied by a decrease in the blood sugar concentration. The injection of 0.1 cc. anterior pituitary extract (Cornish: Parke, Davis and Co.) results in a relatively great rise in the blood sugar level with no detectable change from the normal glycogen content of the liver.

While glycogen is regularly present in considerable amounts in the livers of 7- and 8-day embryos, it is present in but traces on the twelfth day. When the blood sugar level is raised by the injection of glucose, no increase in glycogen content is evident in 12-day livers.

2. THE EFFECT OF HUMIDITY ON THE DEVELOPMENTAL RATE OF CHICK EMBRYOS INCUBATED UNDER INCREASED ATMOSPHERIC PRESSURE. Bert Cunningham, Duke University. (Lantern; 10 min.)

Previous studies had shown an acceleration of growth under pressure as compared to controls, the temperature and relative humidity being constant in both pressure and control incubators. It was noticed, however, that the control eggs lost water more rapidly than the experimental and it was therefore suggested that the retention of water might be responsible in part for the accelerated growth. Other workers had already observed that increased humidity, within rather wide limits, increased the growth rates of chick embryos.

To test this hypothesis, the humidity of the experimental incubator was lowered until the water loss of the experimental eggs was comparable to that of the control eggs. When these adjustments had been made, a setting of forty experimental eggs at 25 pounds pressure, and thirty-three controls (matched with experimentals for weight) was made. After 11 days of incubation the embryos were then removed, stripped of their membranes and weighed. The average water loss in the control eggs was 1.8 gm.; in the experimental 2.0 gm. The average weight of the controls (33) was 3.69 gm.; of the experimental embryos (31) it was 6.07 gm., an increase of about 65% over the controls. When compared to the 42% increase secured in earlier experiments where the relative humidity in control and experimental incubators was kept the same, it would appear that the retention of water prevents the maximum effect of the pressure, and pressure may now be definitely considered as responsible for the accelerated growth.

3. DIFFERENTIATION CAPACITY OF PIECES OF THE CHICK BLASTODERM IN EARLY PRIMITIVE STREAK STAGES. Dorothea Rudnick, University of Rochester. (Lantern; 15 min.)

Chick blastoderms in the early stages of primitive streak formation were cut into three or four pieces and these pieces, with or without entoderm, cultivated on plasma clots for 2 to 6 days. The cuts were made so as to isolate A) anterior material that is to remain uninvginated: i.e., ectoderm; B) material just antero-lateral to the streak, which is to become mesoderm or entoderm depending on the stage of invagination; C) the primitive streak itself. The latter piece was sometimes subdivided into an anterior and a posterior piece.

In these experiments the entoderm does not differentiate and its presence has no effect on the behavior of the upper layer. Ectodermal (A) pieces are capable of forming medullary tissue *in vitro*. B pieces form mainly heart in the earlier stage taken; medullary tissue in later streak stages. Streak (C) pieces form erythroblasts in earlier, heart and erythroblasts in later, stages. These results indicate that localized prospective areas for medullary plate and heart are migrating toward the streak to be invaginated. A general progression in frequency of differentiation of these structures is noted as older blastoderms are tested; other features of progressive organization are also apparent; nevertheless pieces from the earliest streak stages are capable of as perfect histological differentiation as are those from older ones.

4. SELF-DIFFERENTIATION OF THE AXES AND OF THE SKELETON IN TRANSPLANTED LIMB PRIMORDIA OF CHICK EMBRYOS. Viktor Hamburger and Molly Waugh, Washington University, St. Louis. (Lantern; 10 min.)

Wing and hind limb primordia (thickened ridges or small buds) of chick embryos of 52 to 68 hours of incubation were transplanted to the flank or into the coelom of host embryos of the same stage. A considerable percentage developed into limbs of normal shape and almost normal size. The state of determination of the ap- and of the dv-axis was tested by heteropleural transplantations and by rotating the primordium 180°. These two axes proved to be determined at the stage of operation in both wing and hind limb primordia.

The skeletons of the transplants were studied in sections and in whole mounts prepared by the methylene blue method. In most cases, the skeleton of the free appendages approached the normal conditions. Hypodactyly was found in several cases, but other elements were rarely missing. Reduplications were found in few cases and in digits only. All joints were remarkably well developed. Since in many cases the location of the transplant precluded any functioning during development, the formation of articulations must be independent of functional activity.

The girdles were complete and the arrangement of the components was normal in almost all cases. However, the individual elements were frequently abnormal in shape, and the girdles were small in relation to their respective appendages.

5. DETERMINATION OF THE DORSOVENTRAL AXIS OF THE FORE LIMB IN AMBLYSTOMA MICROSTOMUM. Harvey B. Lovell, University of Louisville. (Introduced by A. R. Middleton.) (10 min.)

In order to study the effect of size of graft upon the determination of the dorsoventral axis in *Amblystoma microstomum*, a series of grafts only 2 somites broad have been made and compared to previous experiments which had shown that the axis is determined before stage 33 for $3\frac{1}{2}$ -somite grafts. In all operations the left fore limb bud was transplanted to the right flank with the dorsoventral axis inverted.

At stage 36 the axis was not yet established. In every case a harmonic right limb developed. In two-thirds of the cases at stage 37 the axis was not established, whereas in one-third of the cases it was already determined. Only 19% of the grafts were still unpolarized at stage 38, and 81% had the dorsoventral axis determined before the operation. At stage 39 all cases had the dorsoventral axis irreversibly established. Stages 37 and 38 appear to be transitional stages.

A third series of grafts 5 somites in diameter have been performed at stage 29 with similar orientation. Such large grafts retained their prospective asymmetry and formed inverted limbs.

It appears that the determination of the dorsoventral axis is a gradual rather than a sudden process. There is also evidence that the limb rudiment in *microstomum* is smaller than in *punctatum*. However, the large number of defective limbs obtained (55%) suggests that all of the rudiment was not included in 2-somite grafts.

6. THE EFFECT OF DEAD OPTIC VESICLE UPON EXPLANTS OF PROSPECTIVE ECTODERM. C. D. Van Cleave, University of Pennsylvania. (Lantern; 12 min.)

The influence of dead optic vesicle upon explants of prospective ectoderm of early gastrulae and of body ectoderm from late neurulae of *Amblystoma punctatum* was studied in these experiments, exclusively by the explantation method. An ether extract of dead optic vesicles induced neither lens nor other structures in explanted body ectoderm of neurulae. No inductions were obtained in explants of body ectoderm of neurulae containing one dead optic vesicle. Explanted fragments of prospective ectoderm differentiated as epidermis. Explants of prospective ectoderm containing two to six dead optic vesicles were killed by this

amount of dead material. Seventeen of forty-one explants of prospective ectoderm containing one dead optic vesicle showed inductions of definite neural character after 7 days cultivation. Pattern and organization were lacking in the induced masses; entodermal and mesodermal rudiments were not observed. In three such explants the induced structures consisted of optic rudiments and lentoids connected with a brain mass. The tentative conclusion is drawn that the influence of the dead optic vesicle is imposed on the indifferent ectoderm. The results of similar experiments of Lopashov and of Holtfreter are discussed.

7. EXPERIMENTS ON THE DEVELOPMENT OF THE TAIL AND RELATED STRUCTURES IN URODELA. Paul L. Risley, State University of Iowa. (Lantern; 15 min.)

Implantations of mid-dorsal lip materials of late gastrulae of *Ambystoma maculatum* in the blastocoele of early or midgastrulae of *Triturus torosus*, or in reciprocal combinations, result in the formation of secondary tails on host embryos. Similar observations have been made by Mangold, Bytinski-Salz, and Holtfreter with other species. It is apparent that a capacity for induction of tail region resides in posterior neural, notochordal, and also mesodermal tissues. In several cases, the induced tail shows a well-developed anal gut and anus. Factors involved in their development are of interest, since, normally, the anus is regarded as a remnant of the blastopore of gastrulation.

Amputations of the tail region in *Triturus torosus* were made at about stage 28. Three anteroposterior levels were chosen: one posterior to the anus, one just anterior to it, and one farther anterior. No regeneration of anus or tail follows the most anterior amputation; complete or nearly complete tail development follows the most posterior cut; partial development follows removal of tail bud just in front of the anus. A rounded caudal fin with anus and tail gut develops posterior to the cut, but notochord, neural tube, and somites do not grow into the regenerated region. This suggests that the formation of tail gut and anus is independent of neural tube, notochord, and somites. It is therefore due either to the mesenchymal tissues that succeed in organizing a complete caudal fin, or possibly to the independent elongation of the gut itself.

8. EFFECT OF DIET ON THE SMALL INTESTINE OF *RANA SYLVATICA* LARVAE. Ralph G. Janes, Wayne University College of Medicine. (Lantern; 12 min.)

Linear measurements were made of the small intestine from three groups of *Rana sylvatica* larvae. One group was fed dried boiled beef liver; another boiled lettuce; while the third, which was used as a control, was collected in the field during various stages of development. The animals were killed at 4-mm. increments in body length, beginning at 22 mm. The intestines were the shortest in the liver fed animals, but were about equal in length in the other two series. The animals which were fed liver grew much faster and became larger before they metamorphosed. Because they were larger, the intestines of this group closely approximated the length found in the smaller, but mature larvae of the other two series. During and immediately following metamorphosis, the three groups of animals varied somewhat in size, but their intestinal length was very similar. Thus, the difference found in the lengths of the larval intestines of the three series would seem to be only relative, and apparently degree of differentiation, rather than size, governs intestinal length.

Variations induced by diet in the larval intestines of *R. sylvatica*, did not appear to affect their orientation or coiling, as has been claimed for other species by earlier workers.

9. AUXIN YIELDS FROM FROG TISSUES AND EMBRYOS (*RANA PIPPIENS*). True W. Robinson, University of Illinois. (Introduced by Leigh Hoadley.) (Lantern; 15 min.)

Studies were made of the amount of auxin obtainable from embryos and certain tissues of the adult frog by extraction with acidified chloroform. The auxin yields from the ovaries of pituitary injected and non-injected females were determined. The treated females received two injections, each injection containing two mashed frog pituitaries. The ovaries from both types of females gave about the same yields, although there was a tendency for the ovaries from the injected females to show a lower auxin concentration (per milligram of wet weight) than those from non-injected females.

A comparison of the auxin yield from the ovaries with that from hind leg muscles from the same female indicated a slightly higher concentration in the muscles.

The auxin seemed to be more easily extracted from the ovaries than from the muscles. Similarly, it was more easily extracted from developing tadpoles than from unfertilized eggs.

A study of auxin yield as a function of embryonic development showed that 7-day tadpoles (age from time of fertilization) yielded nearly three times as much auxin per individual as did freshly obtained unfertilized eggs. The auxin concentration per milligram of wet weight of tadpole was about twice that of the unfertilized egg, one-half that of the muscles, and considerably less than that found in previous experiments to be obtainable from the 3-day-old chick embryo.

10. ANALYSIS OF THE DEVELOPMENT OF FISH EMBRYOS BY MEANS OF THE MITOTIC INDEX. V. THE PROCESSES OF EARLY DIFFERENTIATION OF ORGANS IN *FUNDULUS HETEROCLOTUS*. Roy W. Jones, Central State Teachers College, Edmond, Okla. (Introduced by Aute Richards.) (Lantern; 15 min.)

In order to determine the function of cellular multiplication in the differentiation of embryonic organs, counts were made of the resting and of the dividing cells in embryos from critical periods in the early morphosis of *Fundulus heteroclitus*. The basis of analysis was the 'mitotic index' or the percentage of the cells of a region or embryo in division at the time of fixation. Variations in the rate of cell division appear to be correlated with changes in the structure and shape of the embryonic organ. Periods of differentiation or reorganization alternate with periods of cellular proliferation. During stages of differentiation the mitotic index decreases, but it increases during intervals of growth. Mitosis and differentiation are to be considered alternative processes. Growth (increase in protoplasmic volume) of an organ as a whole may occur simultaneously with increased cell division. The degree of morphological development does not correlate necessarily with cell number or age. The mitotic rates reported are more than adequate to supply the increase in cell number from one stage to the next.

The mitotic indices of the organs and the embryos vary, but, as most of the processes of this period of early organ formation include an upward swing of the growth curve, the progressive decrease in the mitotic rate, due to advancing age and differentiation, is not evident.

11. ADAPTATIONS FOR VIVIPARITY IN EMBRYOS AND OVARY OF ANABLEPS ANABLEPS.
C. L. Turner, Northwestern University. (Lantern; 10 min.)

In the Anablepidae, a family of viviparous cyprinodont fishes, the mature eggs are fertilized in the ovarian follicles. The embryos are retained in the modified ovarian follicles until birth and are born when about 44 mm. in length. The embryos develop expanded yolk sacs which reach a maximal diameter of about 10 mm. The vitelline veins on the surface of the yolk sac develop rows of bead-like swellings, yolk sac bulbs, which serve to facilitate absorption of materials from the fluid of the follicle cavity. There is an extensive system of follicular villi upon the internal lining of the follicular capsule, the apparent function of which is to increase the vascular surface of the internal lining of the follicular capsule and to facilitate the interchange of materials between the blood of parent and fluid of the follicular cavity. The follicular villi and the yolk sac bulbs develop at the same time. These two adaptations for viviparity are apparently unique in this family of fishes.

12. CERTAIN ASPECTS OF SWIM BLADDER AND STOMACH POUCH DEVELOPMENT IN HEMICHROMIS BIMACULATA. Robert S. McEwen, Oberlin College. (Lantern; 15 min.)

The swim bladder in *Hemichromis bimaculata* is derived from a solid dorso-lateral outgrowth of gut epithelium. After acquiring a lumen the bladder is lined by columnar cells which become highly vacuolated, completely obliterating the cavity. The epithelium then rapidly flattens, and the lumen reappears filled with gas. It is suggested, in accordance with the general hypothesis of Powers and others, that this gas is produced by the disintegration of materials within the epithelium.

There are two connections between the bladder and the gut, neither of which possesses any lumen at any time. One, the primary connection, is the remains of the epithelial outgrowth which gives rise to the bladder. It comes to consist of a loop, one limb of which is attached to the posterior end of the bladder, and the other to the gut. The latter limb eventually separates from the former, and becomes an extensive stomach pouch. The secondary connection, consisting entirely of connective tissue, is attached to the bladder ventrally and slightly anterior to the primary. At its other end it first connects directly with the tissue about the gut, but later shifts its contact at this end to the tissue about the stomach pouch, or former left limb of the primary.

13. THE UNUSUAL MORTALITY THAT CHARACTERIZES A PARAMECIUM CULTURE. Edgar P. Jones, University of Akron. (Lantern; 9 min.)

That extensive mortality is a normal and recurring phase in populations of *Paramecium multimicronucleatum* has been determined by employing a procedure of counting. Seven cultures have been studied continuously through periods varying between 60 and 100 hours. Each has shown population fluctuations of such an order that the number of paramecia which have died (without a dead body appearing in the culture) within any 3-day period, exceeds the maximum population of that 3-day period. After each depression, a new population upswing takes place.

Variations in the culture turbidity, although not exactly correlated with the population changes, supports the concept.

Cytolysis is the only mechanism which would explain population fluctuations of this magnitude and type. It appears that paramecia which are not at any moment adequately adjusted to the shifting infusion environment are eliminated by bursting. They so become food for their better adjusted associates.

The agents which cause this bursting are sought. Some excretory material is suspected. Tests show that urea, uric acid, or ammonia are not responsible. Since some of my *Paramecium* cultures have lived for more than 4 years, the causative material must be one that does not accumulate. It is recognized that the cell contents might be expected to neutralize the unidentified material, and so to permit another upswing of the population.

It is apparent that *Paramecium* cultures offer an excellent experimental medium for investigation of the theory of the struggle for existence.

14. THE POTENTIAL LONGEVITY OF A PARAMECIUM CULTURE. Edgar P. Jones, University of Akron. (Lantern; 6 min.)

Observations which have been published (University of Pittsburgh Bulletin, 29, 3) led to the conclusion that a laboratory culture of *Paramecium multimicronucleatum* declines because of a lack of food, rather than as a result of a long-time accumulation of a waste product. Consequently, a test experiment was begun.

Fourteen loosely-covered *Paramecium* hay-infusion cultures of 1 gallon capacity were used. Similar controls maintained occasional paramecia for as long as 2 years. Of the experimental cultures, six contained *Paramecium* populations after 30 months. Four of these produced dense populations of paramecia (300 to 400 per cubic centimeter) under the stimulus of frequent feeding at that time. Three cultures maintained paramecia for more than 4 years.

No particular routine was followed in the feeding of the cultures, other than that populations were fed if it was observed that the number of paramecia had materially decreased in one of the cultures. This occurred at intervals which were approximately monthly. The usual food was dry flour, but egg, chocolate, and bacto-peptone were used with success at times. Water was never added.

15. THE RELATION BETWEEN THE NUMBER OF INDIVIDUALS PER VOLUME OF CULTURE SOLUTION AND RATE OF GROWTH IN CHILOMONAS PARAMECIUM. S. O. Mast and D. M. Pace, Johns Hopkins University. (Lantern; 15 min.)

There is still much controversy concerning the question as to whether protozoa secrete substance which augments growth. The more recent work favors the contention that there is no such secretion and indicates that the increase in rate of growth observed, with increase in numbers, is correlated with changes in the character and the quantity of the food available.

Chilomonas paramecium grows readily in a sterile solution which contains only relatively simple chemicals in known concentrations. It is therefore well suited for experiments concerning the question under consideration.

Three methods were used: 1) Different numbers of chilomonads were put into the same quantity (0.4 cc.) of culture solution in depressions on Pyrex glass slides and counted at different times for 24 hours. 2) One chilomonad was put into each of several depressions containing different quantities of solution (0.006 to 0.4 cc.) and counted at different times for 24 hours. 3) Different numbers of chilomonads (1 to 400) were put into the same quantity of solution (0.1 cc.) in different depressions. After 12 hours 0.006 cc. solution from each was transferred to a clean depression and one chilomonad added, then they were counted every hour for 10 hours.

The results obtained with the three methods are essentially the same. They prove conclusively that, within certain limits, as the number of chilomonads per volume of solution increases, the frequency of fission increases, to a maximum of more than 50%, then decreases to zero.

The results obtained in various modifications of these experiments strongly indicate that *Chilomonas* secretes substance which favors growth.

16. THE RELATION BETWEEN HYDROGEN ION CONCENTRATION OF THE CULTURE MEDIUM AND RATE OF GROWTH IN CHILOMONAS PARAMECIUM. S. O. Mast and D. M. Pace, Johns Hopkins University. (Lantern; 10 min.)

Chilomonas paramecium grows well in a solution consisting of $\text{Na-C}_2\text{H}_3\text{O}_2$, 150 mg.; NH_4Cl , 50 mg.; K_2HPO_4 , 20 mg.; MgSO_4 , 10 mg.; CaCl_2 , 1 mg; and H_2O , 100 cc. The H-ion concentration of this solution is pH 7.

A small quantity of this solution, with the hydrogen ion concentration increased or decreased as desired by adding HCl or NaOH, was put into each of a number of depressions on Pyrex glass slides and one chilomonad added to each. After 24 hours the chilomonads were counted and new cultures prepared. This was repeated daily for 32 days.

It was found that as the hydrogen ion concentration decreased, the rate of growth increased from nearly zero at pH 4.4 to a maximum average for four lines of 3.304 divisions per day at pH 6.8 and then decreased nearly to zero at pH 8 and to zero at pH 8.8.

We demonstrated in earlier work that the rate of growth in *Chilomonas* is independent of variations over a wide range in the concentration of chlorine and sodium in the solution used. The results presented were therefore very largely if not entirely correlated with changes in the hydrogen ion concentration, not with changes in concentration of chlorine or sodium.

Loefer ('35) found two optima, one at pH 4.9 and one at pH 7, with a median minimum at pH 6. He used glucose, peptone culture medium. This may account for the difference between his results and ours.

17. THE RATE OF PULSATION AND THE FUNCTION OF THE CONTRACTILE VACUOLE IN *PARAMECIUM MULTIMICRONUCLEATUM*. I. RELATION BETWEEN FEEDING AND THE RATE OF PULSATION OF THE CONTRACTILE VACUOLES. John A. Frisch, Johns Hopkins University and Canisius College. (Lantern; 5 min.)

In mass cultures of *Paramecium multimicronucleatum*, observed daily for 20 to 30 days, simultaneous observation of the rate of pulsation and of the rate of feeding revealed that the average rate of pulsation and the average rate of feeding varied from day to day; but that usually the rate of pulsation varied directly with the rate of feeding, increasing or decreasing from day to day as the rate of feeding increased or decreased. The size of the food vacuoles and of the contractile vacuoles varied with the age of the culture, so that the numerical relation in seconds between the rate of pulsation and the rate of feeding at any particular time depends upon the relative sizes of the food vacuoles and of the contractile vacuoles. The results indicate that variations in the rate of pulsation are due to variations in the rate of feeding, and suggest that the effect of many experimental factors on the rate of pulsation is probably indirect, i.e., they affect the rate of feeding.

18. THE RATE OF PULSATION AND THE FUNCTION OF THE CONTRACTILE VACUOLE IN *PARAMECIUM MULTIMICRONUCLEATUM*. II. RELATION BETWEEN LOCOMOTION AND THE RATE OF PULSATION OF THE CONTRACTILE VACUOLES. John A. Frisch, Johns Hopkins University and Canisius College. (Lantern; 10 min.)

In vaseline enclosures from 1 hour to 30 days old, the rate of pulsation of the contractile vacuoles was lower in active than in resting individuals of *Paramecium multimicronucleatum*; the magnitude of the difference depended upon the kind and the extent of the locomotion. 'Spasmodic movements,' a few seconds in duration, either decreased or increased the rate of pulsation. 'Crawling,' or locomotion, usually in contact with the substratum, between neighboring masses of food, and sometimes for longer distances, decreased the rate and sometimes stopped pulsation. Swimming on a spiral path over relatively long distances greatly decreased the rate and usually stopped pulsation instantly. Observation showed that the relation between locomotion and the rate of pulsation is indirect, i.e., the pulsations decrease in rate or stop during locomotion because feeding decreases or stops during locomotion. Observation also showed that there is a constant absorption of water from the oesophagus during the formation of the food vacuoles, and also when no food vacuoles are being formed; and that this absorption may be interrupted during locomotion. The results indicate that there is no endosmosis of water through the pellicle, and that all the water expelled by the contractile vacuoles enters the animal with the food vacuoles and by absorption from the oesophagus.

19. QUANTITATIVE DETERMINATION OF DEXTROSE IN CULTURES OF COLPIDIUM, GLAUCOMA, CHILOMONAS AND CHLOROGONIUM. John B. Loefer, Berea College and New York University. (Lantern; 8 min.)

Benedict's ('31) colorimetric method was used to determine dextrose utilization by *Colpidium campylum*, *Glaucoma piriformis*, *Chilomonas paramecium* and *Chlorogonium euchlorum*. The organisms were grown in culture tubes in a 1% bacteria-free Bacto-tryptone medium at pH 6.7 containing 0.1% dextrose. The number of organisms per cubic centimeter at the beginning and end of each series was determined by means of a Sedgwick-Rafter counting-cell. Average counts were compared with the number of organisms in controls without dextrose similarly inoculated and incubated at 28°C. Dextrose content of the medium was determined before inoculation and incubation, and again after a short period of growth. *Colpidium* and *Glaucoma* metabolized dextrose in significant quantities. When amount of dextrose consumed by the two species was correlated with the number of organisms (calculations based on logarithmic growth), unit consumption per hour was greater for *Colpidium* than for *Glaucoma*. Growth without dextrose is usually accompanied by alkali formation, but acids produced when sugar is metabolized may more than offset this increase causing a decrease in pH. Neither *Chilomonas* nor *Chlorogonium* metabolized dextrose in significant amounts, although growth with it was apparently better.

20. COMPRESSION STUDIES ON AMEBAS. A. A. Schaeffer, Temple University. (Lantern; 12 min.)

When amebas are gradually compressed between two parallel surfaces, they break at a point with a narrow range, for a given species and given cultural conditions. This index of compression is not related to surface or volume, but to the proportion of ectoplasm to endoplasm.

If an ameba in V-shape is cut into halves so that one pseudopod is retracting and the other expanding, the retracting pseudopod can be compressed to a much greater degree before breaking, than the expanding pseudopod. A piece cut from the anterior end of an ameba breaks under compression before a break occurs in a piece ten times as large, if the latter is cut from the middle or the posterior end or from a retracting pseudopod. *Chaos chaos*, although from 50 to 100 times the volume of *C. diffuens*, can be compressed to within about 30% of the degree of compression required to break *diffuens*.

In *Chaos chaos*, increase in size beyond about 450 μ in diameter, is almost wholly due to increase in the amount of endoplasm; the ectoplasm increases to a much smaller extent. It is incorrect to make the general statement that the ectoplasm and the endoplasm are structures of position, and that they are mutually convertible. Only a part of the endoplasm can be so converted, and in large amebas, only a small part. The rest of the endoplasm is not protoplasm in the sense that ectoplasm is protoplasm.

21. THE RELATIONSHIP BETWEEN THE REACTIONS OF AMOEBA TO LIGHT AND ALTERNATING CURRENT. H. T. Folger, Harold Thomas and Fred Alsup. Fisk University. (Lantern; 15 min.)

Amoeba responds to an alternating electric current, after an appreciable reaction time, by a cessation of movement. The reaction time is dependent upon voltage, increasing as the voltage is decreased. After an amoeba has responded, a certain amount of time must elapse before it will give a similar response to a second stimulus. If the time is short it will not respond at all; if the time is somewhat longer it will respond, but with an increased reaction time. In these and several other respects, the response to alternating current is very similar to the response to light. However, the two stimulating agents cannot have exactly the same effects. If an amoeba has responded to light, and is then subjected to an alternating current, it will again respond, but the reaction time will be considerably shorter than it would have been if the light had not been applied. Also, shortly after having responded to light, the amoeba will react to an electric stimulus that is too weak to elicit a response when used by itself. Thus, light makes the amoeba more sensitive to electricity. Likewise, an electric stimulus makes an amoeba more sensitive to light. Two stimuli, one of light, the other of electricity, and each too weak when used by itself to cause a response, may bring about a response when both are applied, one immediately after the other.

22. PRODUCTION OF AUXIN BY YEAST (*SACCHAROMYCES CEREVISIAE*). True W. Robinson, University of Illinois. (Introduced by Charles Zeleny.) (Lantern; 15 min.)

Pressed yeast is known to contain auxin. In investigations here presented production of auxin by growing yeast was established.

Standard methods were evolved for growing yeast, for measuring growth in terms of cell numbers, cell volumes, and density of suspension, and for determining auxin yield and pH of centrifuged medium.

Tests indicated that chloroform extractions were not necessary since satisfactory determinations of the auxin yield could be made directly on the centrifuged medium.

The amount of growth, auxin yield, and pH of medium were studied as functions of the concentration of Bacto-peptone in the Williams' culture medium. Concentrations of peptone from zero to 10% were used. The following results were obtained. 1) Total growth and rate of growth were greater in the higher peptone concentrations. 2) The greatest auxin yields were obtained from the 0.1% peptone medium, and relatively large yields from the 0.01% and 0% peptone media. Practically no auxin was obtained from media containing more than 0.2% peptone. 3) The more acid the medium the greater was the auxin yield. 4) In a given culture most of the auxin was obtained after the rate of cell division had started to decrease, and the rate of auxin yield per cell increased as the rate of cell division decreased. 5) In cultures growing under different environmental conditions the rate of auxin yield per cell was higher in cultures with a slow rate of cell division than in those with a high rate of division.

23. SOME EFFECTS OF NUMBERS PRESENT ON RATE OF CYTOLYSIS OF EUPLANARIA DOROTOCEPHALA FOLLOWING ULTRA-VIOLET RADIATION. W. C. Allee and Janet Wilder, The University of Chicago. (Lantern; 15 min.)

Planarians were exposed to the full spectrum of an air-cooled quartz mercury-vapor arc and transferred immediately to non-radiated water. The dosage used produced onset of cytolysis usually in from 1 to 3 hours.

With mutual shading during irradiation eliminated, planarians cytolized more rapidly if isolated (into 5 to 10 cc. of water) than if ten worms were placed together in the same volume. This result was obtained a) in well water, b) in distilled water, c) in well water made to $m/120$ $CaCl_2$, and d) in a calcium-free synthetic pond water. There was no significant difference in grouped-isolated survival in a calcium-rich synthetic pond water in which the worms cytolized more slowly than in well water.

The grouped worms also survived longer if placed after irradiation in equal volumes per worm with equal surface area which placed the isolated planarians in quite shallow water. When the depth of such proportional volumes was identical, there was no significant difference in survival.

Planarians irradiated in groups (ten in 10 cc.) and observed singly (one in 5 cc.) survived longer than worms irradiated when isolated (one in 5 cc.) and observed singly after transfer to the same amount of fresh water. Consistent with all the foregoing results, when exposed and observed in similar volumes, worms survived longer if group irradiated and group observed than if singly irradiated and singly observed.

24. RATE OF GROWTH OF DAPHNIA LONGISPINA AS AFFECTED BY FISH-CONDITIONED WATER AND BY CERTAIN FISH EXTRACTS. Lester Ingle and Asher Finkel, The University of Chicago and University of Illinois. (Lantern; 15 min.)

Newly released Daphnia were used and were carried to the time when they themselves released young (end of fifth instar), to assay the relative growth-promoting effects of the following substances: a) goldfish conditioned water, b) mucus extracted from the surface of goldfish in weak alkaline solution, and c) such mucus further purified by precipitation at its iso-electric point. Such materials have been shown by Allee and associates to be growth promoting for goldfish. Definite concentrations of these preparations added to manure infusion media in which the Daphnia were cultured produced, during the first instar, a significantly greater increase in body length as compared with sisters in appropriate controls.

The animals were transferred to fresh medium containing similar additions of the material under assay at the beginning of each successive instar. Growth increments of the experimental animals for the second instar were below those of their respective controls. In the third instar the experimental animals showed an even greater relative increase in size than in the first instar. Growth relations of the fourth instar were like those of the second instar; again a positive response was observed during the fifth instar. Under the conditions of this experiment the added materials produced a relative increase in growth in the first, third and fifth instars, and a relative decrease in the second and fourth instars. The body size of the experimental Daphnia was, at the end of the fourth instar, significantly larger than that of the control animals, and this difference was still greater at the end of the fifth instar.

25. SOME ASPECTS OF THE BEHAVIOR OF THE FRESH-WATER JELLYFISH, *CRASPEDACUSTA* SP. Lorus J. Milne, Randolph-Macon Woman's College, Lynchburg, Va. (Introduced by J. I. Hamaker.) (Lantern; 12 min.)

Many female gonosomes of unusually large size (22 mm. in diameter) studied in lake and laboratory were killed by 30°C. but readily tolerated 24°C. and were only partially inactivated by 9°C. Locomotory contractions one per second yielding upward movement averaging 36 meters per hour were measured. Swimming movements were analyzed and the importance of velar coordination stressed. Reactions to light, to gravity, and to moderate changes in temperature were found irregular or lacking. Ingestion of particles in an eddy at the manubrium tip was suggested as a probable mode of feeding. Water ejected from the bell cavity in swimming causes the eddy concerned, and simultaneously provides for replacement of respiratory water.

26. EFFECT OF DIHYDROANDROSTERONE AND TESTOSTERONE ON THE WHITE LEGHORN CHICK. W. R. Breneman, Indiana University. (Lantern; 15 min.)

Injections of the synthetic male hormone preparations used in this study resulted in a cessation of growth in the chick testis as measured by gonad weight. Treatment was limited to 5 days and series were spaced to cover 5-day intervals from hatching to the twenty-fifth day of age. Comb growth was markedly stimulated and continued after the injections were stopped. Some chicks crowed when 11 days of age. Inhibition of testis was most marked in the 5-, 10- and 15-day series; the 20-day group was only slightly below normal size and the testis of 25-day-old chicks were not significantly different from the controls. Histological examination showed that the seminiferous tubules were normal in size and appearance and that lower weight was due to the failure of the interstitial tissue to increase. The testes of injected chicks were only temporarily inhibited and weight returned to that of normal after injections were terminated.

27. SOME EFFECTS OF PROGESTIN-CONTAINING EXTRACTS AND PROGESTERONE ON YOUNG MALE ALBINO RATS. J. K. Lamar, The University of Chicago. (Introduced by Carl R. Moore.) (Lantern; 9 min.)

Progestin-containing extracts of sow corpora lutea, freed of estrogens, and a synthetic progesterone preparation (Prolution, Schering) were assayed on rabbits by the Corner-Allen technique. Injections of heavy doses (0.5 to 3.0 Rb.U. daily) of these preparations were made into young male albino rats, both normals and castrates. Whereas there was but slight effect from such injections in normal males, there was a definite androgen-like effect on certain accessories in castrates. Males castrated at 25 days of age, then injected for 15 days with 1.0 to 3.0 Rb.U. of progestin or progesterone, showed at autopsy significant weight increases for ventral prostate, preputial gland, periurethral tissue, coagulated gland and seminal vesicle, this in order of decreasing percentage difference from controls. Ventral prostates of injected castrates showed 'light areas' similar to those in the normal controls. Other accessories showed no apparent histological change from the castrate condition.

An attempt was made to assay the androgen-like ability of progesterone against testosterone. Not enough animals were used to test for statistical significance, but on the basis of ventral prostate, preputial and periurethral tissue weights, 1 mg. of progesterone is equivalent to about 0.03 mg. of testosterone in androgenic potency. Other accessories do not respond so well to progesterone. Whether this is a threshold phenomenon or selective action of the hormone it is impossible to say at present.

28. THE GONADOTROPIC AND ADRENOTROPIC CONTENT OF THE PITUITARY GLAND OF THE CHICKEN. Roland K. Meyer, C. H. Mellish and Herbert Kupperman, University of Wisconsin. (Lantern; 10 min.)

The gonadotropic and adrenotropic content of adult male and female chicken pituitaries (pars anterior and pars intermedia) was determined by injecting suspensions of frozen glands in quantities varying from 50 to 900 mg. into hypophysectomized and normal 21- to 22-day-old female rats.

The gonad-stimulating action of pituitaries of each sex was further characterized by determining whether or not its effect on the rat ovary could be augmented by the addition of heme or a human pregnancy urine extract and inhibited by the injection of rabbit serum which contained a sheep pituitary gonadotropic inhibitory substance.

With regard to the gonadotropic activity it was found that the injection of 100 and 500 mg. of male gland into hypophysectomized rats resulted in ovaries weighing 57 and 95 mg., respectively, whereas like quantities of female gland gave ovarian weights of 24 and 75 mg. The results indicate that the male pituitary is somewhat stronger than that of the female.

Glands of each sex had follicle-stimulating and luteinizing action. The gonadotropic effect was augmented by both heme and the pregnancy urine extract but not inhibited by the antigonadotropic serum of the rabbit.

In hypophysectomized rats the smaller doses of male and female chicken pituitary preparations increased the weights of the adrenals over those of the uninjected controls and larger quantities produced adrenal weights which were greater than those of non-hypophysectomized rats of the same age.

29. GONADOTROPIC INHIBITORY SUBSTANCE AND PRECIPITINS IN THE BLOOD OF MONKEYS RECEIVING GONADOTROPIC HORMONE PREPARATIONS. Harold R. Wolfe and Roland K. Meyer, University of Wisconsin. (Lantern; 10 min.)

A study was made of the gonadotropic inhibitory substances and precipitins in monkeys; four were injected with pregnant mare serum having gonadotropic activity, two with highly purified gonadotropic extract of pregnant mare serum, and two others with non-pregnant mare serum. The materials were given in a series of injections at intervals of 30 days or more and during a period of several months.

The initial series consisted of 6.0 rat units given in three injections on alternate days and all succeeding series consisted of 4.5 rat units given in two injections. Three and 1.5 cc. respectively of non-pregnant serum were given per series.

Blood of monkeys which received pregnant mare serum showed fairly high precipitin content and gonadotropic inhibitory substance 10 days after the last injection of the third and subsequent series, but decreased markedly 30 days or more after the last injection of each series. Reinjections restored both substances.

The monkeys injected with non-pregnant mare serum produced precipitins but no gonadotropic inhibitory substance, whereas the blood of the monkeys injected with the purified gonadotropic extract showed gonadotropic inhibitory effects but no evidence of precipitins.

The results show that gonadotropic inhibitory substances can be produced by the injection of relatively small quantities of hormone and also indicate that precipitins are not responsible for the gonadotropic inhibitory effects. The similarity in the time of appearance and disappearance of the inhibitory effect and precipitins offers additional evidence for the concept that the inhibitory substance may be an antibody.

30. AUGMENTATION OF PITUITARY GONADOTROPIC EXTRACTS BY HEME. L. E. Casida, Roland K. Meyer and W. H. McShan, University of Wisconsin. (Introduced by Leon J. Cole.) (Latern; 15 min.)

A quantitative study of the augmentation of gonad weight by in vitro mixture of heme with pituitary gonadotropic extracts was made in immature female rats and monkeys, mature cows and immature male rats and birds. Augmentation of gonad weight was obtained only in immature male and female rats. In the immature female rat, the relationship of the factors of age, heme dosage and pituitary dosage to augmentation was studied.

In general, no difference in augmentation of ovarian weight was noted between 21- and 31-day-old rats. Variation in either pituitary or heme dosage was found to affect the augmentation of ovarian weight. Ovarian size tended to increase as the quantity of either substance was increased. This was true whether total amounts were increased by holding the number of injections constant and increasing the dose per injection, or by holding the injection quantity constant and increasing the number of injections.

It was possible to produce much larger ovaries by the injection of optimum doses of heme and gonadotropic extracts than with any dose of the pituitary extract used alone.

31. THE ACTION OF PREGNANCY URINE HORMONE (PU) ON THE FEMALE GENITAL TRACT OF CASTRATED MONKEYS. Frederick L. Hisaw, R. O. Greep and H. L. Fevold, Harvard University. (Lantern; 15 min.)

Pregnancy urine hormone (PU) was injected in combination with oestradiol and progesterone to determine whether it modified the actions of these two substances on the genital tract of castrated adolescent *Macacus rhesus* monkeys. Each monkey was given 100 RU of oestrin daily for 20 days, preparatory for the experiments in point. When 100 to 300 RU of oestradiol plus 30 to 200 RU of PU were given daily for 30 days, the effects of oestradiol on the uterus, vagina and sexual skin were not altered, but the cervix was considerably larger than when oestradiol was given alone. Metaplasia of the cervical glands, an oestrin effect, was present.

Progesterone inhibits the action of oestradiol on the sexual skin, vagina and cervix when injected simultaneously, but the two hormones act synergistically on uterine development. Oestradiol plus progesterone and progesterone alone will develop a premenstrual endometrium in which the glands become secretorially

exhausted within about 25 days (Hisaw et al., '37). When PU is added to such treatments it greatly prolongs the secretory phase of the glands, but does not modify the effects on the sexual skin, vagina and cervix. The formation, development, and time of involution of decidual plaques resulting from traumatization of the uterus during a progesterone treatment is not influenced by PU; neither does PU postpone bleeding following cessation of injections of oestrin. The pregnancy urine hormone produces an enlargement of the cervix when given with oestradiol and prolongs secretion in the uterine glands when injected simultaneously with progesterone.

32. VARIATION OF GONAD-STIMULATING MATERIAL IN THE HYPOPHYSIS OF MALE GROUND SQUIRRELS. L. J. Wells, University of Missouri. (Lantern; 15 min.)

Individual hypophyses of seventy ground squirrels (*Citellus*) were tested at approximately monthly intervals during two annual sexual cycles. A mouse litter was the experimental unit for these tests. An intramuscular injection of a single hashed hypophysis suspended in saline was made in the leg of an immature female mouse. Seventy-two hours later, recipients and untreated sisters were killed and weighed. A microscopic examination of a fresh vaginal smear was made, and the reproductive tracts (ovaries, fallopian tubes and uterus) were weighed.

Slight sex-stimulating capacity was detectable in the hypophysis of immature males at least 1 month preceding the date of expected spermatozoa.

Hypophyses of adults with spermatozoa showed great gonad-stimulating potency. The same was true of hypophyses from bilateral castrates which were killed during months of low sexual activity for this species.

Hypophyses of normal adults killed during the lowest point (aspermia; involuted accessories) in the sexual cycle exhibited little yet detectable gonadotropic potency. Weight of reproductive tracts from mouse recipients for these animals was 1.23 to 2.57 times greater than that for untreated littermates in eight of nine such tests.

33. FACTORS INFLUENCING THE CHARACTER OF HEAT IN THE GUINEA PIG. William C. Young, Edward W. Dempsey and Carl W. Hagquist, Brown and Yale Universities. (Lantern; 15 min.)

Development of the hypothesis that heat is caused by synergistic action of oestrin and progesterone was followed by attempts to determine what factors regulate its character, i.e., length and extent of mounting activity. A possible partial explanation comes from study of ovaries removed from 221 animals with known behavior records.

Length of heat is not related to the extent of general follicular development provided some growing follicles are present, to rete cyst size provided some ovarian stroma remains, to the number of ruptured follicles provided one ruptures, nor does it change between puberty and the 500th day of life as the general ovarian condition changes. What does seem important, provided a minimum quantity is present, is the manner oestrin is made available when progesterone begins to act.

Mounting activity likewise is not related to general follicular development or rete cyst formation, but in twenty-seven active animals the number of ruptured follicles averaged 3.48 while in thirty-six quiet animals the number averaged 2.70. Barring the possibility of coincidence, this suggests the quantity of oestrin produced by maturing follicles may influence extent of mounting.

Consequently, length of heat and mounting activity appear to be under different types of hormonal control. This hypothesis would explain the complete lack of relationship between length of heat and the extent of mounting noted elsewhere. The possibility is also important because it raises the question if activity would be a valid measure of sexual drive in this species. Experiments suggested by the morphological data are in progress.

34. THE RETICULO-ENDOTHELIAL SYSTEM AND HORMONE REFRACTORINESS. I. EFFECT OF PROLAN ON THE YOUNG NORMAL AND SPLENECTOMIZED MALE RAT. Albert S. Gordon, William Kleinberg and Harry A. Charipper, Washington Square College, New York University. (Lantern; 8 min.)

Young normal and splenectomized male rats (weight 40 to 50 gm.) were given daily doses of pregnancy urine extract (10 R.U. follutein or antuitrin S). At the end of 25 days of treatment, the splenectomized animals showed much more striking effects than the similarly treated controls. Thus heavier testes possessing a more highly hypertrophied and hyperplastic interstitial tissue, and heavier seminal vesicles showing a greater epithelial height were evident in the animals deprived of their spleens. It appears that the greater effects in the splenectomized animals occur because such animals produce a smaller quantity of the antagonistic principle than do the injected controls.

To obtain the above results, it is essential that the animals employed be free of latent infection, especially *Bartonella muris*. If young heavily infected *Bartonella* male rats, with spleens intact, are injected with pregnancy urine extract for 25 days, effects of about the same intensity as those observed in treated *Bartonella*-free splenectomized animals are obtained. Experiments indicate that this is due, most likely, to a partial 'blockage' of the reticulo-endothelial system, caused by the presence of the *Bartonella* organism.

35. THE RETICULO-ENDOTHELIAL SYSTEM AND HORMONE REFRACTORINESS. II. EFFECT OF THYROTROPIC HORMONE ON THE YOUNG NORMAL, SPLENECTOMIZED AND 'DYE-BLOCKED' GUINEA PIG. Albert S. Gordon, William Kleinberg and Harry A. Charipper, Washington Square College, New York University. (Lantern; 7 min.)

Thyrotropic hormone (Collip or Rowlands and Parkes) was injected daily into young normal and splenectomized male guinea pigs for periods as long as 4 months. Three criteria, namely basal metabolic rate, weight of thyroid and histology of the thyroid, were employed. Maximum effects occurred in the normal injected animals about 2 to 3 weeks after beginning treatment; this was then followed by definite regression. In the splenectomized animals, accentuated effects were maintained for about 35 days; this was also followed by a state of refractoriness due, presumably, to reticulo-endothelial compensation. The results were even more marked in thyrotropic hormone injected splenectomized

animals, whose reticulo-endothelial systems were 'blocked' to some extent by repeated doses of trypan blue. Here metabolic rates ranging from 50 to 80% above normal, an increased thyroid weight amounting to as much as 150 mg., and the presence of a heightened follicular epithelium, vacuolated colloid, and interstitial tissue hypertrophy in the gland were observed for as long as 3 months after beginning injections. The pituitary glands of such animals showed a progressive increase in the numbers of basophils. The results are again interpreted as further evidence of the contention that the reticulo-endothelial system is intimately concerned with the development of refractoriness to injected hormone preparations.

36. COMPARATIVE EFFECTS OF LIGHT STIMULATION AND ADMINISTRATION OF GONADOTROPIC HORMONES ON FEMALE SPARROWS. Gardner M. Riley and E. Witschi, State University of Iowa. (Introduced by Frank A. Stromsten.) (Lantern; 15 min.)

Female sparrows were subjected to additional daily periods of light during the fall, winter and early spring. Contrary to the stimulating effect of such treatment on the testes of this bird, the ovaries responded little or not at all. That this non-effect is due to a difference in the responsiveness of the female pituitary to activation by light and not to a refractoriness of the ovary to gonadotropic substances was demonstrated by injection experiments. Injections of beef anterior lobe extracts, water suspensions of acetone-dried turkey pituitary and pregnant mare serum stimulated ovarian development throughout the inactive phase of the sexual cycle. Any kind of gonad stimulation becomes more effective the closer to the normal breeding season it is administered. This indicates an underlying autonomous hypophyseal cycle. Certain hormonal conditions were found to stimulate mainly the growth of the gonads, others the gonads as well as the secondary sex characters.

37. ENDOCRINE AMPHISEXUALITY IN THE FEMALE STARLING (*STURNUS VULGARIS*) Emil Witschi, Richard A. Miller and William Hart, State University of Iowa. (Lantern; 5 min.)

The starling like most other wild birds exhibits yearly cycles of sexuality. A number of secondary sex characters follow closely the rhythm of the gonads. Of particular interest are two which appear in both sexes alike, namely the male gonoducts and the bill color. The female has well-developed vasa deferentia, which form seminal glomi, much as in the male. They enlarge at the onset of each breeding season. The bills in both sexes are of a dark gray, almost black color during the season of sexual quiescence and turn a bright yellow in spring. Castrates of both sexes show the usual shrinkage of the gonoducts and they maintain permanently the dark bill color. Injection of female sex hormones (dihydrotheelin, theelin and theelol of Doisy) cause only an enlargement of the oviducts of the female castrates. Injection of testosterone propionate (Ciba) causes enlargement of the vasa deferentia and color change to yellow in castrates of both sexes. It is therefore concluded that the normal female starling produces male as well as female sex hormones, that both are released in increased quantities during the breeding season and that the secondary sex characters appearing in both sexes are in fact male sex characters.

38. PROLOGATION OF THE LIFE OF THE CORPUS LUTEUM BY HYSTERECTOMY IN THE RAT. James T. Bradbury, University of Michigan. (Introduced by G. R. La Rue.) (Lantern; 15 min.)

The effects of hysterectomy on the ovarian cycle of the rat have been studied. If the ovarian blood supply is not damaged, the removal of the uterus does not affect the estrous rhythm of the mature female. If the hysterectomized rat is mated the resulting pseudopregnancy is longer than that of the normal rat. In a large series of matings the average duration of pseudopregnancy is about 18 days as compared to 12 days for normals and operated controls.

If the uterus is removed from a pregnant rat there is a tendency for the corpora lutea to persist for a length of time approaching what would have been term for that pregnancy. This occurs whether the uterus is removed as early as the sixth or as late as the sixteenth day of pregnancy.

39. OPTICAL STUDIES ON THE STRUCTURE OF PROTOPLASM. Francis O. Schmitt, Washington University.

Attention will be called to the information made available by optical methods concerning the molecular organization and ultrastructure of protoplasm. The methods involved are principally those of the polarization and x-ray optics, together with the built-up film technique recently developed by Langmuir.

40. SOME PROBLEMS ON CELLULAR PHYSIOLOGY AT SUB-ZERO TEMPERATURES. Basile J. Luyet, St. Louis University.

Keeping for a long time living matter in a state of latent life at low temperature and bringing it back to the active state has lured many biologists. The most successful experimental investigations in that line have been those in which the material was dried and brought at some 200° below zero (seeds, spores, encysted protozoa, dried rotifers, tardigrads, nematods). So far as we are aware, all the attempts with tissues containing any appreciable moisture have failed and only single cells like bacteria, cocci, yeast, could stand the temperature of liquid air without being previously dehydrated. We succeeded with epidermal tissues of plants, isotonic with 2% NaCl, having therefore a relatively high water content. Quantitative data have then been obtained on four of the most important factors which control the resistance of a cell to the temperature of the liquid air: water content, cell size, velocity of cooling, heat capacity. Investigations are now on the way for reviving large size protozoa sprayed in liquid air with an atomizer (to reduce the heat capacity) and for reviving muscle fibers (from arthropoda and frog). The probable reason for the resistance of certain forms of protoplasm to extremely low temperatures is the absence of crystallization at these temperatures, a theory held by some physicists and for which the evidence, from biological material observed in liquid air, will be discussed.

41. SOME EFFECTS OF ULTRACENTRIFUGING UPON THE PHYSICAL STRUCTURE OF PROTOPLASM. H. W. Beams, State University of Iowa.

Cells may be subjected to forces ranging from 15,000 to approximately 1,000,000 times gravity in the air turbine ultracentrifuge. In addition to the cellular elements usually stratified it is possible to displace both the mitochondria and the Golgi apparatus. In fact, incomplete evidence indicates that both these elements may be removed from the *Ascaris* egg without killing it. Centrioles and chromosomes have been displaced in chick embryonic cells and fragmentation of the chromosomes and binucleate cells have been obtained in the root tips of wheat.

About 80% of yeast cells budding in a centrifugal field of approximately 150,000 times gravity have their buds originating in a position at right angles to the plane of the stratified materials. This evidence indicates that polarity has been controlled in these cells. *Ascaris* eggs subjected to 150,000 times gravity frequently undergo nuclear division in the centrifuge without the accompanying division of the cytoplasm. Probably this condition is due to a displacement or inactivation of a material that is necessary for the normal cleavage process. Also, *Ascaris* eggs dividing in the ultracentrifuge frequently have their diminution process greatly modified. Furthermore, subjection to 150,000 times gravity for 10 days or to 800,000 times gravity for $\frac{1}{2}$ hour does not kill them. This fact is of interest when comparing the ultramicroscopic structure of protoplasm with that of non-living colloidal solutions. The ultracentrifuge promises to be a useful tool in the study of many problems of experimental cytology and embryology.

42. THE EFFECT OF THERMAL AND ELECTRICAL STIMULATION ON THE VISCOSITY OF AMOEBA PROTOPLASM. C. A. Angerer, University of Pennsylvania. (Introduced by the Secretary.)

In the laboratory, viscosity changes following stimulation have been studied in the case of ultraviolet and mechanical stimulation. As yet no study has been made of thermal or electrical stimulation. The relative viscosity was determined by the centrifuge method. The material studied was the plasmagel (*Amoeba proteus*) and the plasmasol (*A. dubia*). Amoebae were reared in a constant temperature cabinet and upon removal were subjected to various known rates of thermal stimulation within the biokinetic range. The viscosity of the plasmagel was tested from moment to moment until a constant value was attained. Thermal stimuli applied suddenly cause a pronounced decrease in viscosity, followed, in turn, by an increase to a maximum value, after which the viscosity fluctuates and finally levels at a constant value. With slowly applied stimuli over the same thermal range there is a progressive decrement in viscosity, followed by no fluctuation. In the case of electrical stimulation the viscosity of the plasmagel decreases in proportion to the current density and the duration of application. After attaining a specific viscosity value, characteristic for the current density in question, the viscosity levels, upon continued application, until the anodal breakdown occurs on stronger stimulation. A study of the plasmasol is now in progress.

43. THE ELECTRIC CHARGE OF PROTOPLASMIC COLLOIDS. L. V. Heilbrunn, University of Pennsylvania.

There have been numerous investigations of the charge at the surface of living cells, but very few authors have attempted a study of the charge on the colloidal particles of the cell interior. When an electric current is sent through a culture of amebae, as is well known, all the organisms move toward the cathode, and this movement involve a rapid cathodic migration of various cytoplasmic inclusions. It might be urged that the cathodic movement of both cytoplasmic inclusions and the ameba itself is due to a positive charge on the protoplasmic colloids, but there are various alternative explanations possible. If now one makes the amebae alkaline by immersing them in dilute solutions of NH_4Cl , then both cytoplasmic inclusions and the amebae as a whole migrate less rapidly toward the cathode; or even to the anode in over 40% of the cases. The simplest explanation of this phenomenon is that the rise in pH of the protoplasm causes a reversal from positive to negative charge. If one sends a current through a streaming plant cell (*Elodea*), the speed of the chloroplasts toward cathode or anode is influenced by the charge at their surfaces. When the plant cells are studied in the light, the charge at the surface of the chloroplasts may be either positive or negative. However, under conditions in which photosynthesis is prevented or slowed, the chloroplasts show a positive charge. In general, protoplasmic constituents appear to be positively charged. This charge may be reversed by alkalization or by photosynthesis.

44. THE OVULATION PROCESS AND THE MIGRATION OF THE RAT OVUM. J. S. Nicholas and Edgar Allen, Yale University.

The stages preceding ovulation show a definite increase of activity in the cells lining the follicular wall. The process of ovulation has been observed and recorded by moving pictures. The explosive nature of this activity with the subsequent hemorrhage and change in the ovarian follicle are clearly shown.

The migration of the egg has been rechecked in the light of earlier studies and the details have been checked by transplantations. The hydrogen-ion concentration has been correlated with the passage of the egg down the fallopian tube. The uterus is adequately sensitized for the reception of the egg 3 days before the egg has reached the endometrium. Extra eggs have been transplanted after normal implantation has occurred and these occasionally implant successfully in addition to the original egg number. The number of eggs liberated from the ovary is greater than the number of embryos implanting; the number of young born is fewer than the number of original implantation sites.

45. ORIGIN OF THE YOLK SAC IN PRIMATES. G. L. Streeter, Carnegie Institution of Washington, Baltimore.

The early stages of the primate yolk sac, heretofore missing, reveal several unexpected features. The yolk sac does not bud off from the inner cell mass in the form of a solid clump of cells that subsequently acquires a central cavity, as had been supposed. Nor is it at any time an intrinsic part of, or homogeneous with, the gut tract. Instead, the earliest cells of the yolk sac are differentiated

as a thin membrane between which and the gut endoderm there arises a narrow cleft. This cleft becomes rapidly distended and so forms the conjoint yolk sac cavity and gut cavity. This cavity is therefore dual in origin and is bordered on its dorsal part by cells that are to form the gut endoderm, an induced product or migratory element from the embryonic ectoderm. It is bordered on its ventral part by the yolk sac endoderm which is a derivative of the primitive mesoblast and is essentially trophoblastic. The gut and yolk sac are thus different in origin and are always abruptly demarcated from each other. The one becomes a definitive part of the embryo and the other is an auxiliary organ to be discarded at birth. It is to be noted that the yolk sac does not function as a container, but as a specialized nutritional membrane. Compared to other forms the yolk sac in Primates remains a simple structure and consequently there are fewer complications to obscure its derivation and early developmental history.

46. THE MAMMALIAN FETAL MEMBRANES AND PLACENTA; THEIR DEVELOPMENT, EVOLUTION AND PHYLOGENETIC SIGNIFICANCE. H. W. Mossman, University of Wisconsin.

The anatomy and development of the various types of placentation will be outlined. The sequence of ontogenetic stages in the higher types of chorio-allantoic placentae is; primitive epitheliochorial, endotheliochorial, hemochorial and hemendothelial. Either of the last three may represent the mature stage. A 'syndesmochorial' stage is practically absent, and forms with interstitial implantation usually also lack the epitheliochorial stage. The syndesmochorial placentae of ruminants are extremely specialized epitheliochorial types and are in no sense an intermediate step between the epitheliochorial and endotheliochorial varieties. Modern epitheliochorial placentae are probably the result of secondary simplification of a primitive endotheliochorial condition by a process akin to arrested development. Primitive epitheliochorial placentation may not exist in modern Eutheria.

Within orders, for instance Rodentia, the fetal membranes may range from relatively primitive to highly specialized types. Evolution ordinarily affects all stages, but may cause marked advancement in early steps with only negligible changes in the mature membranes. The reverse is rare. A classification of Rodentia based on specialization in their membranes agrees closely with those based on anatomical and paleontological criteria.

Evolution proceeds much more slowly in the fetal membranes than in the bodies. Because of this conservatism, membrane characters are especially valuable criteria of relationship between larger groups such as orders and families. Although seriously limited, our knowledge of the membranes is sufficient to substantiate most of our present conceptions of mammalian phylogeny, and to add valuable information about some uncertain groups such as the bats, armadillos, anteaters, sloths and lemurs.

47. THE DEVELOPMENT OF REFLEXES IN THE MAMMALIAN FETUS. Davenport Hooker, University of Pittsburg School of Medicine.

Mammalian fetuses exhibit reflexes elicitable by stimulation or occurring spontaneously. Three theories regarding the sequence of appearance of reflexes exist: 1) That the first reflexes are non-specific and total, in the sense that all of the neuromuscular mechanism functionally present participates. With the further origin and maturation of neuromuscular connections, specific reflexes emerge from the total response. 2) That specific reflexes appear first, fuse into a total type of response, from which specific reflexes again emerge. 3) That specific and total reflexes develop simultaneously, each having its own sequence.

Since there appears to be a 'common denominator' to the development of reflexes in various forms, though each species has a characteristic type of reflex behavior sequence, more refined methods of eliciting reflexes, greater care in eliminating complicating factors (anesthesia, handling, temperature changes, etc.), and a vastly greater knowledge of the differences between fetal and adult physiology must be evolved and used. The great mass of raw data now available on the sequence of mammalian fetal reflex activity requires extension along lines of greater refinements of technic and unbiased synthesis. The latter is probably the more important, at this time.

48. EXPERIMENTAL STUDIES OF THE FACTORS CONTROLLING THE ESTABLISHMENT OF TRANSMISSIVE NERVE-MUSCLE CONNECTIONS: OCCURRENCE OF HYPERNEUROTIZATION. William Beggs Fort, The University of Chicago. (Introduced by Paul A. Weiss.) (15 min.)

'Motor' nerves of adult toads were inserted into the proximal one-fourth of sartorii muscles—nerve free portion (P)—or into the nerve containing distal one-fourth (D) (abbreviated: insert.), with (T) or without (I) transection (abbreviated: transect.) of the original nerve supply. The m. sartorius was denervated, 25 or more days preceding (1), simultaneous with (2), or 40 days or more after (3) implantation of the foreign nerve. The presence or absence of neuro-muscular transmission was tested for by electrical stimulation of the nerve. Transmissive connections were detected by the twenty-third day (after insert.) in PT1 and DT1, by the thirtieth day (after insert. and transect.) in PT2 and DT2 by the twenty-fourth day (after transect.) in PT3 and, with only a few muscle fibers in DI somewhere within 40 days. Transmissiveness was never found in PI. Tensions developed in DT2 (torsion wire myograph) indicated hyperneurotization (multiple innervation of single muscle fibers) to vary about a mean of 4.2% (ten cases). Histological examination of normal m. sartorii has revealed hyperneurotization of an occasional fiber.

These observations indicate: 1) Hyperneurotization of a minor degree is present normally and after the introduction of a nerve into a denervated muscle. 2) Hyperneurotization does not result from the introduction of a nerve into an innervated muscle. 3) The 'condition' of a muscle controls its receptivity to innervation and the rate of neurotization. 4) A time analysis of three component processes in neurotization viz.: nerve outgrowth, nerve-ending formation and receptivity of muscle fibers is feasible.

- 48A. CONDUCTION OF EXCITATION IN VERTEBRATE SMOOTH MUSCLE. Emil Bozler, Ohio State University. (Introduced by Hans O. Haterius.) (Lantern; 12 min.)

Evidence is given that excitation can be conducted from cell to cell in visceral muscles of vertebrates and that many properties of this type of muscle can be understood by assuming that it is a syncytium like heart muscle. Strips of mammalian uteri, under suitable conditions, react to weak electric stimuli like single nerve, or striated muscle fibers. On making a current a response is elicited at the cathode. The contraction is conducted over the whole muscle and obeys the all or none law, showing a perfect analogy with the properties of cardiac muscle. However, quantitatively the characteristics of excitability of smooth muscle are different from those of other types of muscle and from nerve. The chronaxie is several tenths of a second, the refractory phase lasts for several seconds and the rate of conduction is only 0.1 to 5 cm. per second, depending on temperature and the species from which the muscle preparation is obtained. The experiments show that the excitatory phenomena invertebrate smooth muscle are not fundamentally different from those of other excitable tissues.

49. RODS AND CONES IN THE RETINA OF *FUNDULUS HETEROCLOTUS*, AND THE REGIONS OF THE RETINA RELATED TO THE DIFFERENT CHROMATOPHORIC RESPONSES. Earl O. Butcher, Hamilton College. (Lantern; 15 min.)

The upper 70% of the *Fundulus* retina contains rods, and single and double cones. The lower region has only rods and double cones. The double cones greatly resemble twin cones but are definitely not twin cones. In addition to the difference in cone distribution a specialized crescentic region, containing more double cones and rods than any other part of the eye, is found only in the lower region.

Fundulus adapts well to yellow when the color is below, but there is slight, if any, adaptation when the color is above. Since only single cones are in the upper region, it would seem that they are possibly associated with the mechanism of yellow adaptation.

Experiments on covering the eye, rotating the eye, rotating the fish, destroying either the lower or upper region of the eye, and stimulation from above or below on different backgrounds all definitely show that the upper region of the eye of *Fundulus* is related to the lightening of the fish and the lower portion to the darkening of the body.

50. THE CONTROL OF COLOR CHANGES IN THE DOGFISH, *MUSTELUS* BY NEUROHUMORS. G. H. Parker, Harvard University. (Lantern; 15 min.)

The adult smooth-dogfish, *Mustelus canis*, when blinded, takes on a dark color. Blinded pups of this species are also dark irrespective of a white or a black illuminated environment. Blinded pups in darkness are paler than blinded pups in the light. The darker tint of the pups in the light is due to a pituitary secretion, for it is replaced by full blanching when the pituitary gland is removed. In blinded pups the pituitary gland does not discharge intermedin when the fishes are in the dark; it does discharge a very small amount when they are in the light. The receptor for this light response was not discovered. Blanching in

Mustelus can be artificially excited by adrenalin. There is no evidence that it is naturally excited by any blood-borne agent such as the W-substance hypothesized by Hogben. Blanching in *Mustelus* is due to a nerve-produced lipohumor, by a loss of intermedin from the blood, and by anoxemia. The crude concentrating lipohumoral extract from *Mustelus* after a year's retention in the dried state is still effective as a blanching agent for this dogfish. It induces darkening in *Ameiurus* and *Rana*. This rather remarkable response may be due to another substance contained in the crude extract than that which caused the blanching of *Mustelus*.

51. THE RELATIONSHIP OF THE PARS TUBERALIS TO MELANOPHORE RESPONSE IN FROGS (*RANA PIPIENS*). A. L. Soderwall and F. R. Steggerda, University of Illinois. (Lantern; 6 min.)

Hogben and Slome ('31) in a comparative study of frogs and toads, pointed out that failure of the latter to respond to a white background following removal of the anterior lobe of the pituitary gland is due to the accompanying removal of the pars tuberalis. This does not necessarily occur in the frog because of an anatomical separation of the two structures.

Recently Parker and Scatterry ('37) advanced an 'unihumoral' mechanism theory of melanophore control, having demonstrated that blood serum from dark adapted frogs causes melanin dispersion, whereas no corresponding pallor was found when serum from light adapted frogs was injected.

With these two views in mind it seemed logical that if the pars tuberalis could be removed and a permanently black frog produced, the effect could be attributed to an inability of the pars tuberalis to function.

This was done by a delicate cauterization technique, the results showing a definite and permanent melanin dispersion. Subsequent histological examination showed a destruction of the pars tuberalis with no apparent injury to the rest of the pituitary gland.

The results support the theory that the pars tuberalis enables the animal to respond to a white background.

52. THE LIGHT RESPONSE OF *CLITELLIO ARENARIUS* (O. F. MÜLLER). D. E. Minnich, University of Minnesota. (Moving pictures, lantern; 15 min.)

Clitellio arenarius is a marine tubificid worm, 4 to 5 cm. long, living gregariously in the sandy substrate under small stones between the tide levels. Near the Mount Desert Island Biological Laboratory where these experiments were carried out, this species is abundant in a number of localities. Isolated individuals respond to a sudden increase of illumination of sufficient amount by a muscular contraction which begins at the posterior end of the body and progresses anteriorly. The degree of response depends on the strength of the stimulus and varies from a slight movement of the extreme posterior end to a vigorous contraction in the course of which the posterior two-thirds or more of the body is wound into a compact coil. The reaction time of dark adapted animals varies with the light intensity from ca. 2 seconds to ca. 0.7 second. The reaction time is composed of two parts, a sensitization period during which the light must shine and a latent

period which is independent of light. The latter is markedly affected by temperature. Light adapted animals placed in the dark show a typical process of dark adaptation as indicated by a progressive decrease of reaction time with the duration of time spent in the dark. The process of dark adaptation is also markedly influenced by temperature. The results on this annelid closely parallel the results obtained by Hecht with *Ciona* and *Mya*.

53. EFFECT OF LESIONS OF THE CORPUS STRIATUM ON THE BROODING BEHAVIOR OF CICHLID FISHES. G. K. Noble, American Museum of Natural History. (Lantern; 15 min.)

Lesions of the corpus striatum produce apparently a permanent defect in the brooding behavior of cichlid fishes. Nineteen months after the operation a *Hemichromis bimaculatus* which had bred twelve times since the operation still showed defects in behavior. Two others approximately a year after the operation exhibited defects although one had bred twenty-eight times in that period and the other twelve times. Other *H. bimaculatus* and *Tilapia macrocephala* tested for shorter periods invariably showed a modification of the brooding behavior after striatal lesions.

Brooding behavior consists of a) guarding and fanning of the eggs with synchronization of the parents in these duties, b) removal of the recently hatched young to depressions made by the parents, c) distinguishing young of other species from young of their own kind, and d) attracting the young with fin movements. Striatal lesions may produce defects in one or more of these components of brooding behavior. Large lesions tend to involve more components but the same defect is produced by lesions in different parts of the striatum.

Ablation of the distal third or half of the cerebellum of *Tilapia* leads to a marked deficiency in the plasticity of movement but nevertheless the synchronization of the parents at egg laying and the buccal brooding following it remains unaffected. In striking contrast striatal lesions induce failure to synchronize, and defects of brooding in the parents.

54. MOVEMENT OF STREAM FISH. R. V. Bangham and N. L. Bennington, College of Wooster and Beloit College. (15 min.)

The selected stream areas include in addition to the mile of Sugar Creek reported by Bangham ('37) two other locations in clean headwater streams of the Muskingum River system having a somewhat different fish population. From eight to fifteen visits were made during the spring and summer of 1937 to the three areas. Each time the same mile was seined, the larger fish tagged, measured, scales taken, then the fish released. Each time chemical tests, temperature, turbidity and depth readings were taken and samples of minnows and crayfish obtained from a smaller area.

Of the 486 fish tagged from these areas, 65 or 11.3% were retaken on the same mile of stream. Nine fish were retaken twice after being tagged. The recoveries as well as total numbers taken showed much variation from week to week with differences in water depth and turbidity of the streams. Sixty-one, 13 and 12 rock bass were tagged in the three areas with 19.6, 30.7 and 16.6% recovery,

respectively. Sixty-one, 77 and 19 small-mouthed bass were tagged with 19.6, 13 and 31.5% recovery, respectively, while 50, 27 and 8 common suckers were tagged with recoveries of 4, 11 and 25%, respectively. There were 32, 26, and with retakes of 9.4, 11 and 0%, while 8, 0 and 53 red horse suckers showed the following percentage of recovery, 0, 0 and 1.8, respectively.

55. THE ASPHYXIAL OXYGEN CONCENTRATION FOR THREE SPECIES OF FISH. James L. Wilding, Indiana University. (Introduced by Fernandus Payne.) (Lantern; 10 min.)

A series of eighty experiments were made in the attempt to find the oxygen threshold for three species of fish. The reactions of 360 fish were studied, and two methods were used. In the first method, the fish were placed in partially closed battery jars, and the oxygen was removed by their respiration. At the first evidence of asphyxiation, samples of water were tested for dissolved oxygen, hydrogen ion concentration, carbon dioxide and temperature. The accumulation of waste products from the fish may have added to the effects of low oxygen.

In the second series of experiments, the fish were placed in flasks containing tap water. The tap water was then replaced by a continuous flow of water low in oxygen. The oxygen, temperature, and pH were measured at the beginning of the experiment and at the time of asphyxia.

The oxygen concentration at asphyxia was approximately the same in both types of experiments, although there was a wide variation in the duration of the experiments, the pH, and a slight variation in temperature. The dissolved oxygen threshold, as indicated by these experiments, is 2.0 p.p.m. for the yellow perch (*Perca flavescens*), 3.0 p.p.m. for the silver side minnow (*Notropis whipplii*) and 3.25 p.p.m. for the blunt-nosed minnow (*Hyborhynchus notatus*).

56. THE HISTORY OF A PELAGIC POPULATION. Alfred C. Redfield, Harvard University. (Lantern; 15 min.)

A population of small specimens of the pteropod, *Spiratella retroversa* (Lima-cina), appeared in the eastern part of the Gulf of Maine in December, 1933. From collections made during the following 9 months information was obtained showing that the population was a homogeneous one, that its members grew to maximum size in 5 months, declining in numbers as they did so. A second population of small individuals appeared in the Gulf in late spring, originating chiefly from offshore, but in part appearing to be offspring of the original population. These were unsuccessful in maintaining their numbers throughout the summer.

In addition to the information on the life history of *Spiratella*, the data show the rate of drift of the water in its circuit of the Gulf. It supplies also suggestive information on the dispersal of organisms through the lateral mixing of water. It emphasizes the dependence of pelagic organisms upon the current systems of the ocean and the difficulty involved in maintaining a permanent population in any one locality.

57. FAUNA OF YUCATAN CAVES. A. S. Pearse, Duke University. (Lantern; 15 min.)

During summer of 1936 twenty-seven caves were studied. These were 2-cycle solution and fault types. Water temperatures ranged between 22° and 27.2°C.; air, 21.9° to 27.9°C.; humidities, 90.2 to 100%. Over 300 species of animals were collected; seventy-two have been described as new by various authors. About a tenth of the animals encountered were troglobites, a fifth were troglaphiles, and seven-tenths were troglaxenes. Important troglobites were shrimps, isopods, millipedes, chelonithids, spiders, collembolans, crickets, ants, a blind brotulid fish and a blind symbranchid eel. Judged by relationships these were apparently derived from sea, fresh water and land. Some species of troglobites, such as certain millipedes, show the effects of isolation and are more or less restricted to particular caves or regions; others are wide-ranging in Yucatan, especially those crustaceans which live in ground water.

58. EFFECTS OF SALIVARY GLAND ABLATION ON YOUNG ALBINO RATS. J. C. Plagge, The University of Chicago. (Introduced by Carl. R. Moore.) (Lantern; 6 min.)

Bilateral removal of submaxillary and major sublingual glands or ligation of their ducts in newborn or week-old albino rats results in rapid loss of body weight and death in 2 to 7 days. Little or no food is taken after salivariectomy as is indicated by completely empty stomachs, and apparently the animals die of starvation. Bilateral removal of submaxillaries, major sublinguals or both glands from one side does not interfere with normal growth and development. Total salivariectomy on day fourteen or later is not fatal, although the rate of gain in body weight is less than that of operated littermate controls.

Ligation of any duct leads to degeneration of its gland. Duct ligation of one gland in a control animal causes a gradual appearance of degenerative changes that do not become pronounced until at least 6 days after operation. Therefore evidences of degeneration do not appear early enough to indicate a causative relation to death of animals completely salivariectomized.

Forced feeding of cow's milk at frequent intervals directly into the stomachs of rats, after complete removal of both submaxillary and major sublingual glands at the age of 3 days, leads to gain in body weight. These animals begin to feed normally at less than 3 weeks of age and require no further forced feeding.

A consideration of these data suggests that salivariectomy interferes perhaps with the mechanical side of feeding rather than the physiology of digestion or an endocrine imbalance.

59. RAT PROSTATE AND SEMINAL VESICLE GRAFTS IN RELATION TO THE SEX AND SEX HORMONE STATE OF THE HOSTS. Dorothy Price, The University of Chicago. (Lantern; 15 min.)

It was reported earlier (Price, '36) that the prostate gland of a rat castrated at birth continued growth and differentiation and reached a normal secretory state which was maintained up to approximately 35 days when regression began. The seminal vesicles did not continue development. To analyze further these

somewhat unexpected findings by studying the development of the glands in different hormonal conditions, prostate and seminal vesicle tissue of varying ages was transplanted into young and old hosts—normal and castrated males, normal, spayed and pregnant females. One hundred and eighty-nine grafts were recovered. Young prostate tissue from males less than 30 days old survived and possessed diagnostic cell 'light areas' in normal young or old males for months; in normal young or old virgin females until the tissue was 57 days old; in pregnant females until it was 79 days; and in young castrated males up to a tissue age of 30 days (only two cases). Prostate tissue from animals older than 30 days was histologically normal only in older male hosts.

Grafts of young seminal vesicles recovered from young and old normal males had normal histological structure. One graft in a female kept repeatedly pregnant for 5 months showed indications of androgenic stimulation. Older seminal vesicle tissue gave positive grafts only in older normal males.

The survival of histologically normal prostate grafts in pregnant and non-pregnant females indicates the presence of androgenic substance in the female rat. Further work on the problem is now in progress.

60. A SPECIFIC ACTION OF THE ANTERIOR PITUITARY ON THE INTESTINE. J. P. Schooley, O. Riddle and R. W. Bates. Carnegie Institution. (Lantern; 10 min.)

Data obtained on hypophysectomized pigeons demonstrate that in these animals the anterior pituitary supports the development and maintenance of intestinal tissue; this response is identified as an action of the hormone prolactin. The length (a), empty weight (b), and villus length (c) of intestine in groups of birds (total 170) were measured after various treatments. Ten days after hypophysectomy the value of (a) decreased (18%) approximately in proportion to the decrease in body weight (17%); but (b) was reduced by 41% and (c) by 25%. Unfractionated A.P. extracts, as injected during this period, increased body weight by 12% but increased (a) by 23%, (b) by 56% and (c) by 42% over the corresponding values found in unoperated controls. Highly purified and heated prolactin increased body weight by 8%, but it increased (a) by 15%, (b) by 10%, and (c) by 17%. Pituitary preparations free of prolactin and containing probably all other A.P. hormones failed to maintain body weight and permitted equivalent or proportionately greater decrease of intestinal tissue.

Appetite, as measured by food consumed, is greatly influenced by the particular hormone injected. Food intake alone (forced feeding or complete fasting for 10 days) very markedly affects the maintenance of intestinal tissue, but the hormonal factor is nevertheless evident. Thus, operated birds injected with prolactin each consumed 28 gm. daily and unoperated controls consumed 36 gm.; but the former group, despite the loss of their anterior pituitaries and a reduced food consumption, increased their intestinal tissue by percentages stated above.

61. THE EFFECT OF OVARIAN HORMONES AND SEASONS ON THE THYROIDS OF BOTH SEXES OF *ANOLIS CAROLINENSIS*. L. T. Evans, Montana State University. (Lantern; 15 min.)

I. Nine young males and ten young females received subcutaneous injections of aqueous theelin (maximum dosage, $11\frac{1}{2}$ r.u.) during December. Ten male and thirteen female controls of similar weights were used for comparison.

The height of acinar cells average $5.41\ \mu$ in treated males, $4.65\ \mu$ in untreated males, $4.94\ \mu$ in treated females, and $4.69\ \mu$ in untreated females. Thus, treated males showed 14% increase in cell hypertrophy, while treated females showed 6% increase. Treated thyroids of both sexes showed small acini with frothy or vacuolated colloid, while the acini of controls were large with clear darkly-staining colloid which was but slightly and peripherally vacuolated.

II. Thyroids of nine untreated normal males killed in June showed consistently large acini with very low epithelial cells (average height, $3.10\ \mu$) with clear unvacuolated colloid.

Thyroids of five females castrated 3 months previously and killed in June showed clear colloid and acinar cells averaging $3.34\ \mu$ in height. Thyroids of eight untreated normal females killed in June revealed an apparent relationship to ovulation. Three, with mature ova in ovaries or oviducts, showed acinar cells averaging $7.11\ \mu$ in height. The other five were without mature ova; in these the acinar cells averaged $3.44\ \mu$.

It seems evident that seasons and ovarian hormones affect directly or indirectly the activity of the lizard thyroid.

62. STUDIES ON THE PHYSIOLOGY OF THE AVIAN THYROID. Dorothea Starbuck Miller, State University of Iowa. (Introduced by E. Witschi.) (Lantern; 10 min.)

It was found previously (Miller, '35) that injection of crystalline thyroxin into male sparrows causes modifications in feather color in the direction of female type plumage. Attempts have been made to duplicate these effects by producing hyperthyroidism through injection of thyrotropic hormone. Changes in thyroid histology and in metabolic rate have been studied as criteria of the hyperthyroid condition. Although the glands are highly stimulated and the metabolic rate is elevated by treatment with thyrotropic hormone, the regenerated plumage is completely normal. The explanation for these negative results is being sought by feeding and injection with various thyroid preparations. No refractoriness in the response to thyrotropic hormone has been noted. After injections over a period of 10 weeks, the glands were enormous and highly activated; the metabolic rate was 17% above normal.

Seasonal variations in metabolic rate and thyroid histology of normal male and female sparrows have been followed throughout the year. The seasonal cycle is related chiefly to the temperature of the environment, rather than to phases of the reproductive cycle. During the winter, the thyroids are activated; the metabolic rate is correspondingly high. With the approach of warm weather the thyroids become less active and the metabolic rate progressively diminishes. All metabolic rate determinations have been made at a constant temperature of 28°C . The daily cycle in metabolic rate will also be considered.

63. TESTICULAR FUNCTION IN HYPERTHYROIDISM. George K. Smelser, Columbia University. (Lantern; 15 min.)

Administration of thyroxin or dried thyroid gland to adult male rats causes a decrease in testis weight, sperm production and accessory weight. Testicular function is affected by amounts of thyroxin too small to produce weight loss, although larger dosage has a proportional effect. Daily injection of small amounts of thyroxin does not markedly affect testicular structure but the accessories of such animals are like those of castrates, and sperm production, determined by counts of the sperm in the epididymes, decreases markedly.

If thyroxin treatment is discontinued a rapid and complete recovery ensues. Normal testicular and accessory function may be maintained in hyperthyroid animals by injection of gonadotropic preparations. There is a marked decrease in effectiveness of gonadotropic hormones (glandular or urine extracts) in hyperthyroid immature males, which involves both gonadotropic actions though the evidence on the gametogenetic factor is more conclusive. Similarly, the amount of testis hormone required, to stimulate the accessories of castrate rats, is increased approximately five times in hyperthyroid animals. The gonadotropic hormone content of the blood of castrate male rats is not affected by thyroxin injections, which cause marked body weight loss. There is no evidence indicating either a subnormal production or secretion of gonadotropic hormones, or an increase in the rate of their excretion or destruction in hyperthyroid animals. Hypofunction of the male reproductive tract in hyperthyroidism may be explained by an increase in the threshold of response to gonadotropic and male hormones.

64. A BLOOD SUGAR INCREASING EFFECT OF PARATHYROID EXTRACTS. Oscar Riddle and Louis B. Dotti. Carnegie Institution. (Lantern; 5 min.)

Unpublished results which identify prolactin as the 'diabetogenic' pituitary factor provide special present interest in derivatives of other endocrine glands also capable of increasing the blood sugar. It is found that extracts of the parathyroid glands (parathormone, parathyroid extract, Lilly) regularly increase the blood glucose of normal, thyroidectomized, and hypophysectomized pigeons at 15 to 22 hours after injection (25 to 150 redefined units); at 5 hours after injection no response was obtained.

Both fasting and non-fasting birds of both sexes respond to the extract. In fasting rabbits the response is slight or absent at the single dosage (150 newer units) tested. Concurrent measurements of the serum calcium showed this value increased by 28% in pigeons and by 20% in rabbits; the dose level employed was therefore not excessive, and the preparations used were potent. Blood glucose was increased in normal non-fasting pigeons by 26%; in fasting normals by 19%; in fasting thyroidectomized by 8%; in hypophysectomized by 26%. The results are based upon sixty-four blood glucose measurements made on animals injected with two different parathyroid extracts prepared and tested in 1934 and 1937.

65. THE ENDOCRINE BASIS FOR THE RISE IN BLOOD CALCIUM ASSOCIATED WITH EGG LAYING IN THE FOWL. Margaret Altmann and F. B. Hutt, Cornell University. (Lantern; 15 min.)

Injection of egg yolk alone, oestrone in either of two forms, or of yolk with extract of whole anterior pituitary caused a significant rise in the serum calcium of non-laying immature fowls, and also a change in the histological structure of the parathyroid glands, which was altered to resemble that in parathyroids of actively laying fowls. Injection of egg yolk also raised the blood calcium of capons. Extract of whole anterior pituitary injected without yolk did not affect blood calcium, while the thyrotropic principle of that gland decreased the serum calcium significantly. It is considered that the normal rise in serum calcium associated with egg laying results from accumulation of oestrone in the developing yolks, and direct or indirect stimulation of the parathyroids by that hormone. This is supported by the fact that removal of the three largest yolks from laying hens was followed by a drop in blood calcium, not found in operated controls. Evidence from other experiments suggests that the oestrone may stimulate the anterior pituitary to produce the parathyrotropic principle which activates the parathyroid and thus induces the rise in calcium.

66. THE EFFECTIVENESS OF THEELIN IN NORMAL AND CASTRATED ADRENALECTOMIZED FEMALE RATS. Robert L. Kroc, Indiana University. (Lantern; 15 min.)

Following adrenalectomy and marked weight losses, fifteen normal and twelve previously castrated rats were treated with various amounts of theelin. Considerable variation was found in the degree of response as determined by vaginal smears and, in some cases, by autopsy and histological study of the uterus and vagina. This variation seems to be correlated only in part with the degree of adrenal insufficiency. It was possible, however, to produce a full four plus reaction in animals castrated as long as 3 months and with weight losses up to 30%. Positive smears were obtained at death in some cases.

It is concluded that theelin is effective in producing vaginal and uterine changes characteristic of oestrus and that extreme adrenal insufficiency does not inhibit the action of theelin.

67. THE SEPARABILITY OF HYPOPHYSEAL LACTOGENIC AND CORTICOTROPIC ACTIVITIES. Warren O. Nelson and Charles E. Tobin, Wayne University and Yale University. (Lantern; 12 min.)

Considerable difference of opinion exists concerning the relation of the lactogenic and corticotropic hormones of the anterior pituitary, many observers indicating that the two hormones are identical. Evidence from certain experiments on the factors concerned in lactation convinced us that such is not the case. As an outgrowth of these findings four preparations of lactogenic and two of corticotropic extract have been assayed simultaneously for their content of each hormone.

The test subjects were pseudopregnant rabbits for lactogenic hormone and hypophysectomized rats for corticotropic activity. The following table summarizes the results of these assays.

	<i>Lactogenic activity</i>	<i>Corticotropic activity</i>
Lactogenic A	Positive at 10 mg.	Negative at 90 mg.
Lactogenic B	Positive at 15 mg.	Positive at 30 mg.
Lactogenic C	Positive at 15 mg.	Negative at 90 mg.
Lactogenic D	Positive at 30 mg.	Positive at 60 mg.
Corticotropic E	Negative at 80 mg.	Positive at 20 mg.
Corticotropic F	Negative at 80 mg.	Positive at 30 mg.

The dosages given in each instance represent total amounts injected during the test periods. It is apparent that there is no relationship between the lactogenic and corticotropic activities of the various preparations. For example, lactogenic A has high lactogenic activity, but no demonstrable corticotropic potency while lactogenic D, of much weaker lactogenic potency, had fair corticotropic activity. At the levels tested on the rabbit the corticotropic preparations showed no lactogenic potency. It is probable that they would have shown some activity if the more sensitive pigeon crop-gland test had been used.

These experiments offer evidence for the separability of the pituitary lactogenic and corticotropic activities.

68. POST-ADRENALECTOMY DIURESIS AND RELATED CONSIDERATIONS. Robert Gaunt, Hugh E. Potts and Eleanor Loomis, Washington Square College, New York University. (Lantern; 15 min.)

Some have reported a diuresis and others an oliguria in adrenalectomized rats. We found that after adrenalectomy food intake invariably dropped (average 13.5 to 8.3 gm./day); water intake generally dropped (average 21.1 to 19.7 cc./day); urine volume generally, not invariably, rose (average 7.00 to 10.6 cc./day). There was never a negative water balance. In animals developing acute adrenal insufficiency quickly, the drop in food and water intake was greater, the polyuria less than in chronic cases. Initial diuresis and terminal oliguria were characteristic. Rats surviving adrenalectomy showed a chronic mild anorexia, thirst and diuresis.

The diuresis in the rat caused thirst, the water intake being greater than expected from the food (and salt) intake.

Low salt diets caused changes in water exchange in normal animals similar to adrenalectomy. On this diet adrenalectomy diminished the water intake and output but the water/urine ratio was not significantly altered.

In normal rats a 0.9% NaCl drinking solution produced thirst and diuresis, with the only significant change after adrenalectomy being a slight anorexia.

A 0.1% KCl drinking solution produced a slight increase in water intake and urine volume, and after adrenalectomy, a consistent diuresis and reduced life span.

Doses of cortical extracts (Upjohn's, or Schieffelin's charcoal adsorbate) which maintained life satisfactorily must be increased two to four times to eliminate completely all symptoms. Doses which completely restored appetite, growth and vigor sometimes left chronic disturbances in water exchange.

69. THE EFFECT OF THE REDUCTION OF ADRENAL TISSUE ON THE RESPONSE OF THE ALBINO RAT TO COLD. S. M. Horvath, F. A. Hitchcock and F. A. Hartman, Ohio State University. (Lantern; 10 min.)

Studies of the respiratory metabolism of normal and unilaterally adrenalectomized albino rats have been made by means of an open circuit apparatus at environmental temperatures of 29° and 4°C., and at 29° after exposure to 4°C. for periods of from 2 to 72 hours. In all some twenty-four rats were studied. The results justify the following conclusions: 1) the basal heat production of the albino rat is unchanged following the removal of one adrenal; 2) normal albino rats after exposure to an environmental temperature of 4°C. have an average increase in heat production measured at 29°C. of about 22%. The corresponding increase in heat production of unilaterally adrenalectomized animals is only about 7%. 3) At an environmental temperature of 4°C. the maximal heat production of unilaterally adrenalectomized rats was attained more slowly and was lower than that of normal animals. 4) The colonic temperature of both normal and singly adrenalectomized rats remains practically unchanged at environmental temperatures of both 4° and 29°C.

70. STUDIES ON LIVING CONJUGANTS OF *PARAMECIUM CAUDATUM*. Ralph Wichter-man, Temple University. (Motion picture; 15 min.)

The problem of sexuality in *Paramecium* is approached by a new method by placing a single pair of recently joined paramecia in a precision microcompressor. Nuclear division can be observed in the living condition, and time relationships during conjugation established. The conjugants are prevented from spiralling, but are allowed to move around slowly. The behavior of the micronuclei during all three divisions has been observed in the living condition, and a motion picture of the entire process is being made. The micronucleus of each conjugant is seen to increase in size as it leaves the macronucleus. This enlargement takes approximately 4 hours, while the anaphase and telophase stages require 18 minutes. The 'crescent' prophase stage and the long anaphasic-telophasic separation spindle are clearly shown. The swollen part at the center of the separation spindle is passed into the cytoplasm where it degenerates. Each product of the first division enters into the second, where two long spindles are visible in each conjugant. The anaphase and telophase stages take but 9 of the 50 minutes required for completion of the second division. Three products of the second division are seen degenerating. The remaining product enters into the third division, resulting in two pronuclei. The pronuclei of a given conjugant have been seen to fuse and form a syncaryon in the same individual. Contrary to general accounts of conjugation, no evidence has been obtained to show that 'crossing over' of pronuclei takes place in the two races investigated.

71. NUCLEAR DIVISION IN *CHAOS CHAOS* LINNAEUS. M. Catherine Hinchey, Temple University. (Introduced by D. H. Wenrich.) (Lantern; 15 min.)

Chaos chaos (type A of Schaeffer), the giant amoeba which was first seen by Roessel in Germany in 1755 is very like the common laboratory amoeba, *Chaos diffuens*. *C. chaos* is, however, multinucleate, all the nuclei dividing at the same

time. In interphase, each nucleus is discoid, averaging 16μ in diameter, with granules arranged in a layer underneath the nuclear membrane. When division begins, the nuclei become spherical, with the granules in an inner hollow sphere. Next the granules condense, forming a broad band in the equatorial plane. Further condensation produces a lens-shaped mass at the nuclear center. Some of the granules, which will eventually disappear, now move away from the center of the lens-shaped structure leaving behind a row of other granules arranged on spindle fibers along the equator. The nuclear membrane now seems to disappear. In optical section, an equatorial view of the following stage looks like a ladder with a central group of granules arranged along the centers of the rungs. These central granules next separate into two groups which move toward the poles in deeply staining, narrow bands. The spindle fibers extending beyond the bands form polar caps. Next the spindle fibers disappear. Now, in late telophase, the daughter nuclei of the living animal are extremely thin discs. Cytoplasmic division takes place at this time. As reorganization proceeds the discs gradually thicken to form the interphase nuclei. The nuclear events were followed in the living organism under a microcompressor, and in stained material.

72. CANNIBALISM AND GIGANTISM AMONG THE PROTOZOA. A. C. Giese, Stanford University. (Introduced by C. V. Taylor.) (Lantern; 15 min.)

Cannibalism has been reported among many protozoans and in some instances gigantism was observed to follow. In the present study cannibalism was induced in *Stylonychia curvata* and *Blepharisma undulans* by appropriate manipulation of the food. The cannibals became much larger than controls. Such gigantism was demonstrated to be due to 1) increased growth, not inhibited division; 2) an increased supply of food, not to a species specific accelerative substance.

73. THE ORGANIZATION OF THE PARABASAL APPARATUS OF CERTAIN POLYMASTIGOTE FLAGELLATES. Harold Kirby, Jr., University of California. (Lantern; 15 min.)

The parabasal apparatus of Devescovininae consists of one proximal element, alone or with one or more attached, usually cord-like distal elements. In reproduction, at first only the proximal element is present. Distal elements, if any, grow out from this. The proximal element coils around the axostyle in some genera, not in others. Often it is not attached to the blepharoplast at its extreme end; the parabasal filament separates, passing directly to the blepharoplast, while the main substance continues anteriorly against the nuclear membrane beyond the point nearest the blepharoplast. In genera with a coiled proximal element, one or more distal elements occur in one of twenty *Devescovina*, one of thirteen *Meta-devescovina*, one of six *Caduceia*, none of five *Macrotrichomonas*. A distal element may occur in some individuals of certain species usually lacking it. In genera with the proximal element not coiled, distal elements occur in two of sixteen *Foaina*, all of three *Pseudodevescovina*, and in three monotypic genera, namely, *Parajoenia* and two new genera. In one species of *Pseudodevescovina* the distal structure originates in one to three trunks, subdividing into nine to sixteen cords; in another the one to five outgrowths usually present are distributed evenly along the proximal element. In a new genus established for '*Devescovina*

hilli' there are one or two distal elements attached in such manner that the apparatus has the form of a T or cross. In the other new genus the single distal element is a voluminous, compressed structure, not cord-like, turned around the axostyle.

74. OSMIOPHILIC BODIES IN THE CILIATE, *TILLINA CANALIFERA*. John P. Turner, University of Minnesota. (Lantern; 10 min.)

The cytoplasm of *Tillina canalifera* contains three types of inclusions which can be seen in the living cell and demonstrated by several techniques.

The largest and most spectacular inclusions are refractive rods, 2 to 4 μ long, 0.5 μ thick, which appear black after treatment with the methods of Mann-Kopsch and Ludford, dark gray after toned Da Fano, and are negative to Champy-Kull and to intravital neutral red. They are evidently Golgi or Golgi-like elements.

The second type consists of granules 0.2 to 0.4 μ in diameter and dumb-bells up to 1.5 μ long which appear red after Champy-Kull, and Ludford followed by fuchsin; gray or black after toned Da Fano; and bright red with intravital neutral red. These are evidently mitochondria or mitochondria-like elements.

The third type consists of fat droplets, 1 μ or less in diameter, which stain bright orange with Sudan III and react somewhat like 'vacuoles' to osmic impregnation.

Regaud's method is unsatisfactory for *Tillina*. The Kolatchev method as modified by Nassonow gives a striking picture of large granules, dumb-bells and thick rods up to 2 μ in length. This picture is incompatible with that seen in the living cell and brought out by the other techniques. Gruber and Hollborn's Janus green does not stain any part of the cell in intravital preparations.

75. INTRACELLULAR PARASITISM IN FISH PRODUCING A GIGANTIC GROWTH OF THE INFECTED CELLS. Richard Weissenberg, Washington University, St. Louis. (Introduced by Roscoe R. Hyde.) (Lantern; 15 min.)

As in earlier researches of Weissenberg, it has been shown fish cells can be stimulated to a gigantic growth by intracellular Microsporidia (*Nosema*) or in other cases by intracellular organisms of ultramicroscopic size (*Lymphocystis virus*).

By artificial infection of young sticklebacks (*Gasterosteus aculeatus*) with *Nosema anomala* the increase in size of the infected host cells could be definitely traced in different stages. Wandering connective tissue cells hypertrophy here from an initial size of 8 μ in diameter to multinucleated cysts of 4000 μ containing millions of spores.

The *Lymphocystis* disease of fish is characterized by the formation of peculiar reticulated inclusion bodies in the cytoplasm of the uninucleated fish cells which hypertrophy to a gigantic size, for instance, in the European flounder to a diameter of 2000 μ . In collaboration with Dr. Ross F. Nigrelli (New York Aquarium) and Dr. George M. Smith (Yale University) a new case of *Lymphocystis* disease was discovered in the New York Aquarium on a hogfish (*Lachnolaimus maximus*) caught on the coast of Florida. The type of the inclusion bodies is similar to the type of the numerous inclusion bodies in the *Lymphocystis* cells of the European flounder (*Pleuronectes flesus*). But the inclusion bodies of the *Lachnolaimus* cells are of a more compact shape and seem to increase in numbers by budding.

76. GROWTH OF THE INTRACELLULAR SYMBIONTS OF THE COCKROACH, *PERIPLANETA AMERICANA*. H. T. Gier, Harvard University and Ohio University. (Lantern; 10 min.)

The intracellular symbionts of *Periplaneta americana*, and of several other species of cockroaches, could not be cultivated on any type of media devised. They could be maintained, with the tissues containing them, in tissue culture for several days but in no instance was any multiplication of symbionts observed. Implantations of the symbionts into crickets and into chick embryos were unsuccessful.

The total number of symbionts in a growing roach approximately doubles during each instar, i.e., the number increases directly with the weight of the animal and geometrically with time. The rate of increase is relatively much slower around the young oocytes, maintains a constant level throughout the growing stages, comes to a standstill in mature individuals, and retrogresses during old age. Starvation or insufficient diets regularly cause a decrease in the total symbiont number, while treatment with heat, cold, x-rays, ultra-violet light, or mild germicides produce no appreciable change in numbers.

77. A STUDY OF THE CILIATED EPITHELIUM OF THE GILL OF *NUCULA CASTRENSIS*, HINDS. S. I. Kornhauser and Dorothy Blumer, University of Louisville. (10 min.)

The living gill showed the direction of the beat of the various types of ciliated cells and their action in movement of the food particles, in rejection of debris and promotion of respiratory currents. Cellular details and mitochondria were studied supravitaly with neutral red, toluidin blue and Janus green. Bouin material mounted in toto gave valuable information concerning the number and arrangement, size, and surface shape of frontal, latero-frontal, and frontal cells in each gill leaflet. Flemming and Champy material sectioned gave cytological details. Each type of ciliated cell was studied, viewed from above, viewed laterally (at right angles to the effective beat of the cilia), and viewed frontally (in the axis of the effective beat). This permitted reconstruction of the cells giving size, shape and position of nuclei, arrangement of cilia, their number, their connection with the basal bodies, and the form of the intracellular fibrillar apparatus. The cells of *Nucula* were compared with those of *Ostrea*. Each type in *Nucula* bears a general resemblance to the corresponding type in *Ostrea* but the lateral cells in *Ostrea* are more highly differentiated as shown by their shape, their arrangement in definite rows, the variation of the diameter of the cells from row to row, and the arrangement of the basal bodies within the cells.

78. SOME VARIATIONS IN CERTAIN STAGES OF LIVING GERM CELLS OF INSECTS. W. J. Baumgartner, University of Kansas. (Motion picture; 15 min.)

One marked variation in living germ cells is the manner in which the pseudopodia project from the poles during the anaphase and telophase stages of division. Some projections are hair-like, others are broad and pointed, still others are bubble-like. In some set-ups they appear much earlier in the division stages and persist longer than in others. In most cases the pseudopodia are on or near the poles, but in a few instances they were far off to the side of the cell.

Slight differences in the nutrient solutions made up from day to day and changing metabolic conditions in the active cells account for some of these variations, but most of them are specific, that is, every species of insect has its characteristically behaving protoplasm.

79. DETERMINATION OF POLARITY IN THE *FUCUS* EGG BY CENTRIFUGING. D. M. Whitaker, Stanford University.

When recently fertilized eggs of *Fucus furcatus* are centrifuged 15 or 20 minutes at 3000 xg., the visible cell inclusions are thrown centripetally. Most eggs remain visibly stratified until long after rhizoid protuberances form. When reared at 15°C. in the dark, 93 to 99% of the eggs which remain stratified form the rhizoid protuberances at the centrifugal pole ($\pm 10^\circ$), even after being embedded in random positions in agar-sea water before centrifuging, and regardless of the position in which the eggs are held during development. The developmental polarity therefore appears to be determined by the axis of centrifugation.

Stratified materials redistribute in a smaller proportion of the eggs. More eggs undergo redistribution when held in agar with their heavy sides uppermost. The determination of polarity is lost in these eggs, and the determination therefore appears to correlate with the distribution of visible materials.

Essentially similar results have been obtained when eggs are reared in the dark in thin cultures after being ultra-centrifuged in sea water at forces up to 200,000 xg.; when centrifuged at 150,000 xg. for periods ranging from 5 minutes to an hour; and when centrifuged for 5 minutes at 150,000 xg. beginning at different times after fertilization, ranging from 15 minutes to many hours.

All experiments were carried out in sea water initially at pH 7.8 to 8.1, and all eggs were at least 5 to 10 egg diameters apart.

80. DEVELOPMENT OF EGGS WITHOUT NUCLEI OR CHROMOSOMES. Ethel Browne Harvey, Princeton University.

Parts of eggs without nuclei are obtained as previously described, by centrifuging the eggs until they break into two parts; the heavy half is always non-nucleate. After treating these non-nucleate halves with parthenogenetic agents a normal fertilization membrane is thrown off, a monaster forms, then an amphister and often cleavage comes in between the two asters. In *Arbacia punctulata*, cleavage follows upon cleavage in a fairly regular manner until a blastula of some 500 cells is formed. Stained sections of these developing eggs show well-formed asters often in pairs, but no chromosomes nor nuclei. The Feulgen reaction which is specific for chromatin is entirely negative. So far all attempts to get these non-nucleate eggs to develop further than the blastula have proved fruitless.

Several other species of sea urchin, occurring at Naples, have been studied. *Arbacia pustulosa* gives results similar to the *Arbacia* of Woods Hole, but the cleavages have not gone as far. In the other three species, *Paracentrotus lividus*, *Parachinus microtuberculatus* and *Sphaerechinus granularis*, the nucleus goes also always to the light pole, but in *Paracentrotus* and *Parechinus*, the mitochondrial granules are in the heavy half. The non-nucleate halves of all the species can be activated, throw off normal fertilization membranes and start to cleave, but

the cleavage is more irregular than in *Arbacia*. Quite regular looking blastulae are eventually formed, but no further development has taken place. It seems possible that nuclear material is necessary for further development after the blastula.

81. INTERFACIAL PHENOMENA BETWEEN OIL DROPS AND CYTOPLASM. M. J. Kopac, Washington Square College, New York University.

Various interfacial effects are produced whenever living cells coalesce with oil drops. No visible effects result from pure mineral oil but oleic acid causes partial or complete cytolysis in all sea urchin eggs so far investigated. Oleic acid drops quickly become coated by a thick, firm granular layer. Fish liver oil drops injected into starfish oocytes remain perfectly smooth and spherical, but if the cells are cytolyzed (by puncturing their germinal vesicles with a microneedle) the oil drops become heavily wrinkled and deformed by interfacial films of a plastic solid, non-granular nature. The most significant interfacial phenomenon is the lowering of the tension at the oil drop's surface at the instant contact between it and cytoplasm is established. A method based on the change in rate of flow before and after coalescence of an oil drop, driven out of a micropipette under constant pressure, permits an approximation of the magnitude of tension changes at its surface. The tensions of both mineral oil and oleic acid (initial tensions, 45 and 10 dynes, respectively) drop to low values ranging from a fraction of a dyne to only a few dynes. All these effects indicate that cytoplasm possesses a high degree of surface activity at oil interfaces. Since the tensions at oil-protein interfaces are very low (Danielli and Harvey, '35), it is probable that the highly hydrated protein molecules are the chief cytoplasmic components responsible for these surface activities.

82. COLLOIDAL CONDITIONS OF THE NUCLEUS. William R. Duryee, Washington Square College, New York University. (Motion picture.)

The normal nucleus is not a liquid, but a highly elastic colloidal gel, in which both chromosomes and nucleoli are suspended. A wide variety of reagents shows the sign of the charge on the dispersed phases to be negative, with the matrix well on the alkaline side of an isoelectric point between pH 4.0 to 4.8.

In isolated frog germinal vesicles (900 μ average diameter) liquefaction or flocculation may readily occur according to the following rules:

1. Monovalent cations (K and Na) in concentrations below 0.04 M and hydroxyl ions liquefy the substrate allowing chromosomes and nucleoli to sink to the nuclear membrane.

2. Polyvalent cations (Ca, Mg, Hg, Cu and basic dyes) more concentrated than 0.001 M cause a quantitative phase separation involving de novo formation of the 'linin' reticulum. Chromosomes are concurrently denatured and contracted.

3. Hydrogen ions flocculate only at the isoelectric point and liquefy at higher concentrations (film demonstration).

Of particular significance in death changes is the fact that reactions similar to 2 are caused by disintegrating egg cytoplasm (= autofixation) as well as by fixatives, e.g., Bouin, Zenker, osmic acid and alcohol sublimate. It is concluded that no fixative yet tried can produce an accurate microscopical picture of the nucleus.

83. THE CORRELATION OF SEA WATER TEMPERATURES AND SOLAR RADIATION WITH THE PHYTOPLANKTON CALENDAR AT FRIDAY HARBOR, WASHINGTON. Lyman D. Phifer, University of Washington. (Introduced by J. E. Guberlet.)

Four consecutive years of observations (January 1933 through December, 1936) are presented as weekly mean values of daily sea water temperatures, of daily solar radiation as measured by an Eppley thermoelectric pyrliometer, and of the phytoplankton calendar, determined by the centrifuge method as the number of marine diatoms per liter. The annual cycle of each series is discussed with attention to maxima and minima range and fluctuations from the general trend.

Since previous work has shown that the concentrations of phosphorus, nitrogen and silicon rarely, if ever, limit the phytoplankton production, an attempt is made to correlate the time of the annual spring growth of phytoplankton with the sea water temperature and the total solar radiation received. Usually within 10 days after the sea water temperature rises above 9.0°C., the quantity of phytoplankton begins to increase very rapidly. It appears that the solar radiation requisite for phytoplankton is not the limiting factor for the commencing of the spring production, but rather it affects the phytoplankton indirectly in raising the sea water temperature. After the initial outburst, the phytoplankton development is rather irregular, usually reaching its peak before the highest sea water temperature or the greatest solar radiation for the year occurs. The season's production usually terminates before September, during which the annual decrease of sea water temperatures begins, the decrease in solar radiation usually having begun in early August.

84. PHOTOSYNTHESIS IN RELATION TO LIGHT PENETRATION INTO LAKE WATERS. W. M. Manning, University of Wisconsin. (Introduced by C. Juday.)

Solar radiation penetrating into a lake influences photosynthesis directly by supplying the energy necessary to reduce CO_2 to carbohydrates, and indirectly by its effect on water temperature. At high light intensities high temperature is necessary for maximum rate of photosynthesis, while at low light intensities low temperature is more favorable.

Measurements with many species of plants show that low intensity light is used much more efficiently in photosynthesis than light of high intensity. Experiments indicate that, if *Chlorella*, were equally distributed throughout a lake the first 90% of visible light absorbed would produce only about half the total photosynthesis occurring in the lake, with the other half being produced by the last 10% of light to be absorbed. Even under very favorable conditions, it is probable that less than 20% of light energy actually absorbed by *Chlorella* can be converted into chemical energy as carbohydrate.

With respect to energy absorption by chlorophyll, the changing wave-length distribution reduces the effectiveness of light penetrating into a lake, since the wave-lengths most strongly absorbed by chlorophyll are usually removed in the upper water.

The various plankton algae are similar in their reaction to light intensities found at different depths in lakes. Non-plankton plant species showed marked differences in their behavior toward light, especially at high intensities.

Determination of distribution and concentration of chlorophyll in several Wisconsin lakes, together with light intensity and photosynthesis studies, make it possible to estimate approximately the fraction of light incident on a lake that is actually converted and stored as chemical energy.

85. THE RELATION BETWEEN DIATOMS AND COPEPODS AS A FACTOR IN THE PRODUCTIVITY OF THE SEA. George L. Clarke, Harvard University and Woods Hole Oceanographic Institution.

A review is undertaken of recent plankton investigation in which the production and distribution of diatoms and copepods has been studied. The new theories, emanating from these investigations, which have been advanced to explain the interrelations of the plant and the animal plankton are discussed. Laboratory experiments on the nutrition of copepods are also summarized. Besides work carried on at Woods Hole, the contributions to this general problem of Marshall, Nicholls and Orr, of Wimpenny, Harvey, Hardy and Steeman Nielsen are considered. For further work in this field emphasis is laid on the necessity of taking into consideration the time for development, the action of currents, and particularly the nutritive requirements of copepods not only at the time of spawning but also throughout the whole period of growth.

86. THE PRESENT STATUS OF WORK ON THE ECOLOGY OF AQUATIC INSECTS AS SHOWN BY THE WORK ON THE ODONATA. Clarence H. Kennedy, Ohio State University and Franz Theodore Stone Laboratory. (Introduced by Raymond C. Osborn.)

The work on the Odonata has been chosen as a sample because; 1) the speaker is familiar with the literature on this group and, 2) the adults and larvae of dragonflies are better known than are those of any other equally large group. Some of the points discussed are the necessity of exact identification of species in all stages of growth, the problems of hybrid forms, subspecific forms, physiological forms: More specifically in the field of ecology, the problems of population counts, reproductive ability, parasitism in the egg and other stages, predators of young, of adults, the selection of habitat by the egg-depositing female, distribution of young in the water and of adults in the air.

Ecological studies in the general field of entomology as bearing on studies of the ecology of aquatic insects are considered.

87. STUDIES OF THE WATER FLOW THROUGH CERTAIN PELECYPOD MOLLUSCS AND SOME APPLICATIONS THEREOF. T. C. Nelson, Rutgers University. (Motion pictures.)

All pelecypods except Septibranchs feed upon plankton strained from water filtered through their gills. Estimates of volume of water passed have been based upon: 1) comparison of plankton counts of the water and of stomach

contents; 2) rate of clearing of turbid water; 3) degree of deflection of a moveable cone in the path of excurrent flow; 4) actual measurement of water passed through excurrent aperture of the pelecypod. First method defective in loss of plankton incident to feeding. Second method deficient as applied, due to lack of knowledge of effects of turbidity upon the water pumping mechanism, the absence of constant uniformity of suspension, and failure to evaluate effects of increasing viscosity and density of water with falling temperature. Third method of limited value since impossible to calibrate for quantitative results. The fourth method seriously disturbs highly sensitive receptors surrounding excurrent aperture in siphonate forms, but can be successfully used for the oyster. With a modification of the rubber apron of Moore ('08) all water passed by an oyster can be measured without interfering with normal behavior. A maximum of 26 liters per hour with 8 to 12 liters commonly observed in large oyster. Results indicate figures previously determined are low. Maximum hydraulic head developed in oyster, 7 mm. water. Water flow through a pelecypod resembles tide; a large volume under low hydraulic head, hence flow quickly reduced by friction. In evaluation of food intake of pelecypods account must be taken of organisms living on the shell and on bottom.

88. CONTRIBUTIONS OF LIMNOLOGY TO GAME FISH PRODUCTION. William J. K. Harkness, University of Toronto. (Introduced by A. G. Huntsman.)

The movement of game fish, and the movement of plankton and forage fish which constitute their food, in lakes and streams appears to be largely dependent upon the temperature and the oxygen and carbon dioxide content of the water.

The invertebrate fauna on the beds of lakes and streams is definitely characteristic of the temperature, gaseous and flow conditions. The classification of lakes on this basis is a valuable guide to their merits as game fish waters. An analysis of invertebrate fauna of a stream at almost any time gives fairly trustworthy evidence as to the maximum temperature, permanent character of the stream and abundance of fish food.

Analysis of spawning activity is revealing many interesting phenomena. In some waters speckled trout and lake trout appear to spawn annually. In other waters speckled trout appear to spawn intermittently. Lake trout on the other hand appear to reach a spawning maturity and then to enter a period of senescence, when they no longer spawn. These conditions affect the natural egg production and indicate the artificial propagation and planting necessary to overcome spawning deficiencies, and indicate the advisability of introducing strains of the species which spawn annually.

Practically all of the data arising from purely limnological investigations are now being brought to bear upon the determination of the adaptability of water areas for fish production, and are being used as a guide to ways and means of increasing this productivity.

89. A NEW METHOD OF STUDYING THE MOTOR ACTIVITY OF THE STOMACH IN A FISH (*SCORPAENICHTHYS MARMORATUS*). T. L. Patterson, Wayne University and Hopkins Marine Station of Stanford University. (Lantern; 15 min.)

This investigation is a continuation of phylogenetic studies on the comparative physiology of the gastric motor mechanism in the bullhead or cabezone (*Scorpaenichthys marmoratus*), which belongs to the sculpin family (Cottidae).

Through surgical procedure a method was developed for the introduction of a gastric rubber balloon through a stomostomy opening made in the floor of the mouth which did not interfere with the respiratory process. This method also permits of as nearly normal activity and freedom of the fish as could be naturally obtained from such an animal under experimental conditions when placed in a large vivarium provided with running aerated sea water, within which it was possible for the fish to swim about without disrupting the sensitive recording system. Under these conditions the normal activity of the filled and empty stomach was graphically recorded on a slowly moving kymographion by the balloon-manometer method.

Records of digestive and hunger activity were obtained from eight fish in which the stomach exhibited continuous, rhythmical contractions, the intensity of the contractions being greater during digestion. The manometric pressure varied as the fish changed its level in the water and the locomotor activity was also recorded. The gastric contractions during hunger or digestion were inhibited by small quantities of sea water or weak acid and alkali when introduced directly into the stomach.

Studies concerning changes in anatomical structure and physiological function of the alimentary tube during evolutionary development may help to explain the function in higher animal forms.

90. SLEEP; BIOLOGICAL SIGNIFICANCE AND MECHANISM. F. H. Pike, Columbia University. (10 min.)

The nervous system is not an independent variable in the equation defining life, but is concerned in the production of profound changes in the physicochemical substratum. Certain processes proceed at a low rate with no long periods of greatly reduced activity. Other processes at a higher rate have greater alternations of intense and reduced activity. Sleep represents a period of reduced activity of certain high speed processes. The necessity for sleep arises from the inability of a small factory mechanism concerned with slow synthetic processes to provide materials which break down with almost explosive suddenness as fast as a large motor mechanism consumes them. The free energy of the motor system decreases faster than it can be renewed. Enforced reduction of expenditure follows, while synthesis proceeds.

Sleep, to be biologically adequate, must be something which can be entered upon when opportunity offers and terminated suddenly when occasion demands. Experimental evidence of six decades and more shows that the activity of the central nervous system is dependent upon afferent nervous impulses from peripheral organs and surfaces as well as upon internal conditions. The mechanism of sleep lies in reduction of intensity and volume of afferent impulses most closely connected with high speed processes. It is one thing which tends to reduce the total amount of work necessary for maintenance of life.

91. PHYSIOLOGICAL STUDIES ON HIBERNATION IN THE CHIPMUNK. Alvalyn E. Woodward and John M. Condrin, University of Michigan. (Lantern; 15 min.)

In the vicinity of Toledo, Ohio, the eastern chipmunk (*Tamias striatus lysteri* Richardson) appears to hibernate in nature from late November to late February. They may also go into a torpid condition in unheated laboratories during the same season. It is possible to bring about a similar condition in mid-summer by putting them into a refrigerator. This was accompanied by a drop in respiration, heart rate, body temperature and blood sugar.

Seasonal changes in blood sugar were observed, with average values in June and July 60% higher than in January. There were even greater seasonal changes in the weights of the pituitary and adrenal glands, the latter being nearly three times as heavy in summer as in winter.

92. RENAL EXCRETION AT LOW URINE VOLUMES. THE CALCULATION OF 'MINIMAL' UREA CLEARANCES, AND THE MECHANISM OF OLIGURIA. Leon C. Chesley, Margaret Hague Maternity Hospital. (Lantern; 15 min.)

The excretion, at low urine volumes, of urea, creatinine, inorganic phosphate, total nitrogen, and total solids has been studied in normal and nephritic adults, and in patients having toxemia of pregnancy.

When the urine volume falls below 0.4 to 0.5 ml. per minute, the amounts of each substance excreted vary directly and quantitatively with the urine volume. Since the amounts excreted must depend upon the amounts filtered (and therefore upon the volume of filtrate) minus amounts reabsorbed, it is tentatively suggested that the final urine volume varies with the glomerular filtration, at these low levels.

The urine becomes maximally concentrated, under constant conditions, when the urine volume falls to 0.4 to 0.5 ml. per minute. This suggests that a constant proportion of water is reabsorbed.

The excretion of urea, at low volumes, does not obey the square root rule formulated by Möller, McIntosh and Van Slyke. Therefore calculation of 'standard' urea clearances from these volumes is not permissible. It is proposed that a new calculation of 'minimal' clearances be substituted. This is derived by computing what the clearance would be if the volume were 0.35 ml. per minute, below which the urea concentration ratio is constant.

93. THE EFFECTS OF PITUITRIN ON THE WATER METABOLISM IN AMBYSTOMA DURING METAMORPHOSIS. F. R. Steggerda, University of Illinois. (Lantern; 6 min.)

Recently Steggerda has shown that there is a correlation between the natural habitat of the toad, frog and mud-puppy and their ability to absorb water following the injection of pituitrin. Subsequently a comparative study was made of the response of *Ambystoma* to pituitrin before and after metamorphosis.

Six *Ambystoma* (larval stage) weighed in mass, were injected with pituitrin (0.1 cc. per 10 gm. weight) and weighed at half-hour intervals for 7 hours. A week later, after metamorphosis, the experiment was repeated, and again on several occasions thereafter. All the experiments were controlled with similarly treated uninjected animals.

The results show that pituitrin causes *Ambystoma* in the larval stage to increase in weight about 5%, whereas an increase of nearly 10% occurred within a week after metamorphosis and after 2 weeks an increase of approximately 15% was recorded which proved to be the average increase in the adult animal in later experiments.

These results indicate that there is a pronounced difference in the water metabolism of the *Ambystoma* before and after metamorphosis.

94. ADJUSTMENTS OF BODY WATER CONTENTS IN FROGS. E. F. Adolph, The University of Rochester. (Lantern; 10 min.)

Rates at which deficits and excesses of body water were dissipated, were measured at total water contents from +25 to -35% of the control body weights. Each deficit, established by evaporation from the body, was corrected chiefly by more rapid intake through the skin; each excess, established by intraperitoneal injection of distilled water, was compensated by more rapid formation of urine.

Rates of uptake were most rapid at first, diminishing as the deficit decreased, while rates of urine output were more nearly constant until the whole excess had disappeared. Comparison of the water exchanges within the first half hour in which compensation was allowed showed that a given volume of body water in excess was removed more slowly than the same volume in deficit was regained. The coefficients of variation in rates at one content were similar ($\pm 26\%$) throughout the range studied, and were as great in one individual as among individuals.

In deficits the formation of urine ceased; in excesses the intake through the skin continued at undiminished rates. In another series of frogs kept out of water, the same volumes of water in excess were excreted at much smaller rates than in those kept in water.

Put together, the above data describe a kinetic equilibrium in which the frog's water content is inevitably restored to its usual value after each displacement. Only at the control or usual water content does water intake equal water output.

95. EXPERIMENTAL ANALYSIS OF THE VASOMOTOR REVERSAL. Theodore Koppanyi, Charles R. Linegar and Robert P. Herwick, Georgetown University, School of Medicine. (Lantern; 15 min.)

It is a well-known fact that treatment of an animal with ergotoxine or ergotamine produces a fall instead of a rise in blood pressure from the injection of epinephrine. This is spoken of as the 'vasomotor reversal,' and has been attributed to paralysis of the vasoconstrictor sympathetic terminations by the ergot alkaloids.

The experimental analysis of this vasomotor reversal resulted in establishment of the following facts: 1) The type of anesthesia is a primary factor in the elicitation of the vasomotor reversal. Ergotamine tartrate in relatively small doses (0.2 to 1.0 mg. per kilogram of body weight) produces typical vasomotor reversal if the experimental animals (cats or dogs) are narcotized with urethane. Under barbiturate anesthesia, however, ergotamine in the above doses does not reverse but enhances the typical action of epinephrine; and even in larger doses (up to 13.0 mg. per kilogram) does not reverse its action. 2) Ergotamine sensitizes

the cardiac and blood pressure responses following the stimulation of the peripheral and central ends of the vagus nerve. 3) The vasomotor reversal and other actions of ergotamine can be diminished or abolished by suitable doses of atropine. 4) Physostigmine or prostigmin in suitable doses enhances the above effects of ergotamine and conversely ergotamine potentiates the parasympathetic and other effects of physostigmine.

Since ergotamine may be substituted for physostigmine or prostigmin and potentiates their actions, it is concluded that the explanation of the 'vasomotor reversal' as a result of sympathetic paralysis is incorrect and that the ergotamine effects are due to sensitization of the parasympathetic receptor mechanism.

96. A COMPARISON BETWEEN THE BEAT IN THREE AND FOUR CHAMBERED HEARTS WITH REFERENCE TO EMBRYOLOGICAL DEVELOPMENT. Stephen W. Gray, University of Illinois. (Introduced by F. R. Steggerda.) (Lantern; 6 min.)

It is the purpose of this paper to show whether or not there is a difference in the physical nature of the beat in three and four chambered hearts.

To do this, the cinematographic records of the cat's and turtle's heart were studied in particular to determine the change in different dimensions of the ventricles.

The records show that during contraction in the turtle's heart the greatest change is in ventricular length, whereas in the cat's heart the greatest difference occurs in ventricular width. This phenomenon is discussed from an embryological and phylogenetic point of view.

97. COMPARISON OF QUANTITATIVE PRECIPITIN TECHNIQS AS INFLUENCED BY INJECTION PROCEDURES. Joseph G. Baier, Jr. and Harold R. Wolfe, University of Wisconsin. (Lantern; 10 min.)

Rabbits were intravenously injected (3 cc. of undiluted antigen in three injections) and reinjected (2 cc. in two injections) at intervals of one or more months with oxyhemoglobin, crystalline egg albumin and albumin and pseudoglobulin fractions of dog serum. Homologous interfacial test titers and volumetric measurements of precipitates with Van Allen's thrombocytocrits were compared.

Maximum interfacial titers were often produced with one series of injections but at times two were necessary especially with the oxyhemoglobin. This value was not increased by as many as five reinjection series. Measurable amounts of precipitate were produced by two or three series of injections but seldom by one series. With each reinjection series the peak in the precipitate curve shifted to the point of greater antigen concentration and the volume of precipitate increased. This indicated that there was present in the rabbit's blood a larger quantity of antibodies. This increased amount of antibodies may possibly be correlated with the decrease in specificity of an antiserum (previously reported). The contour of the oxyhemoglobin curves was dissimilar to the types found when other antigens were used.

Six monkeys treated similarly but with horse serum proteins responded differently. The interfacial titers were always very weak and measurable amounts of precipitates were not produced.

98. DIFFERENCES IN THE PERMEABILITY OF THE ERYTHROCYTES OF TWO CLOSELY RELATED SPECIES. M. H. Jacobs, H. N. Glassman and A. K. Parpart, University of Pennsylvania and Princeton University. (Lantern; 12 min.)

Characteristic differences in the permeability of the erythrocytes of different vertebrates to dissolved substances have been reported by the authors in previous papers. The present investigation deals in a somewhat more detailed manner with differences between the erythrocytes of two closely related species, the albino rat and the albino mouse. In all the individuals of the two species between which comparisons have as yet been made, ranging in number from three to several dozen of each species for the various tests, an identification of the blood could readily be made by any or all of the following characters: 1) time of hemolysis in 0.3 M erythritol solutions, 2) occurrence of hemolysis in 0.3 M mannitol solutions, 3) time and temperature coefficient of hemolysis in 0.3 M glycerol solutions, 4) effect of traces of copper on glycerol hemolysis, 5) effect of pH changes on glycerol hemolysis, 6) relative rates of hemolysis in 0.3 M glycerol and in 0.3 M thiourea solutions, 7) time of hemolysis in M/6 ammonium borate solutions, 8) retardation by ammonium benzoate of hemolysis in M/6 ammonium chloride solutions, and 9) rate of hemolysis in water after a previous exposure of the erythrocytes to a hypertonic salt solution. These observations give support to the general belief of biologists that specific differences extend to all the cells of the body and are physiological as well as morphological in nature.

99. COLCHICINE TREATMENTS WITH CLADOCERA. H. Howard Dunham, W. Malcolm Reid and A. M. Banta, Brown University and Carnegie Institution of Washington. (Lantern; 15 min.)

The production of numerous genetically identical individuals by diploid parthenogenesis renders Cladocera favorable material for study of drug effects and the detection of hereditary changes. Colchicine in the culture medium at $10^{-3}\%$ concentration for 2 to 18 hours kills *Moina macrocopa* and parthenogenetic eggs laid during such treatment. Most adults treated at $10^{-4}\%$ concentration for 8 to 16 hours survive; most parthenogenetic eggs laid during such treatment develop into viable young. *Daphnia longispina* adults and eggs are somewhat less tolerant of colchicine. Eleven of 246 viable individuals developed from colchicine-treated eggs were sterile. One of the fertile individuals produced a genetically different clone now in its third parthenogenetic generation. None of the controls produced exceptional offspring. Whether this genetic change is chromosomal or genic is unknown.

100. OXYGEN CONSUMPTION AS A FUNCTION OF pH IN THYONE. Wm. A. Hiestand, Purdue University. (Introduced by R. M. Cable.) (Lantern; 5 min.)

Oxygen consumption of *Thyone briareus* behaves as a linear function of pH. Determinations of oxygen consumed were made by the Winkler method using sea water (Woods Hole, Mass.), the pH of which was changed by the addition of HCl or NaOH. The rate of oxygen consumption was determined over a range of pH values from 5.4 to 8.8. The rate of oxygen consumption is reduced at low pH and increased at high pH values within the above range. When oxygen consumption per gram body weight, per hour, is plotted against pH values a straight-line relationship is shown indicating that oxygen consumption in *Thyone* is inversely proportional to hydrogen ion concentration of the surrounding water.

101. ULTRAVIOLET RADIATION OF GRASSHOPPER EMBRYOS (*MELANOPLUS DIFFERENTIALIS*). Malcolm Ray, State University of Iowa. (Introduced by J. H. Bodine.) (Lantern; 8 min.)

The entire spectrum of a quartz mercury vapor lamp was used with controlled temperatures during radiation. The effects were determined through altered oxygen consumption and hatching percentages.

By using eggs when only the chorion is present and by removing the chorion from other eggs it was found that of the chorion and cuticle, only the latter permitted penetration of effective ultraviolet light.

From experiments with dechorionated eggs it appears that brief daily exposures of as little as 1 minute for 20 days reduce the oxygen consumption below 25%, and the hatching below 10% of normal. Single exposures must be longer than 60 minutes to give an equivalent effect. Apparently short daily exposures are more effective than the same total radiation in a single exposure.

A stimulation in oxygen consumption of 10 to 20% was obtained with daily radiations of 15 seconds or single exposures of 15 minutes.

The results of single exposures of 30 minutes at different ages indicate an increasing resistance to ultraviolet light with age of developing embryos. The radiation of first yolk and then embryo in the same egg makes it appear that the effect of the ultraviolet light is entirely on the embryo.

The exposure of normally blocked (diapause) and experimentally blocked (5°C.) embryos to ultraviolet light produced no effect on the oxygen consumption until the embryos became active indicating that the above effects on respiration resulted through action on some intermediate process rather than on the respiratory mechanism itself.

102. ACTIVATION OF NATURALLY OCCURRING ENZYMES. J. H. Bodine, T. H. Allen and E. J. Boell, State University of Iowa. (Lantern; 7 min.)

A detailed quantitative study has been made of the formation, activity and substrate relations of the enzyme tyrosinase in the developing egg of the grasshopper, *M. differentialis*. Enzyme concentration reaches a maximum at 20 days at 25°C. and remains thus throughout the remainder of development. Activation seems necessary for its working since substrate added to naturally occurring enzyme is little affected. Compounds activating enzymes have been discovered and their mode of action investigated. Naturally occurring substrate is found only late in embryonic development. It thus seems that, normally, tyrosinase is formed during ontogeny long before substrate on which it acts is produced. Details of these relations are discussed in light of the so-called 'blocks' occurring during embryonic development.

103. THE HORMONAL FUNCTION OF THE CORPORA ALLATA IN THE GRASSHOPPER, *MELANOPLUS DIFFERENTIALIS*. Isabelle G. Weed, State University of Iowa. (Introduced by H. W. Beams.) (Lantern; 12 min.)

An operative technique has been developed by which the corpora allata, glandular structures occurring in the heads of insects, may be removed from the grasshopper. This operation does not deprive the animal of its ability to eat, move about and copulate.

When these glands are removed from females, the eggs stop development just before yolk is deposited and then degenerate. Likewise, the secretion formed by the oviducts is not produced when the corpora allata are absent. In this respect the oviducts appear to be under the direct influence of the corpora allata since removal of the ovaries does not prevent formation of this secretion when the corpora allata are present. If only one gland is removed, fertile eggs are produced and laid as usual. However, the remaining corpus allatum hypertrophies. Even a fragment of a gland may be sufficient to enable the eggs to complete development, but such a fragment usually increases in size.

It is evident, therefore, that in the grasshopper the corpora allata play an important role in connection with the female reproductive system, probably through the agency of hormones released into the blood. However, to date, no effect on sperm production has been observed when the corpora allata are removed from either young adult males or males in the last nymphal stage; nor does removal of the corpora allata during the last nymphal stage have any effect on moulting.

104. THE GROWTH AND DEVELOPMENT OF THE FEMALE BEE (*APIS MELLIFICA* L.).
R. M. Melampy, E. R. Willis and S. E. McGregor. Bureau of Entomology and Plant Quarantine and the Louisiana State Agricultural Experiment Station.
(Introduced by Wm. H. Gates.) (Lantern; 12 min.)

A physiological study was made of the development of the two female castes of the bee (*Apis mellifica* L.) showing that the queens and workers have approximately the same growth rate during the first $4\frac{1}{2}$ days of larval life, after which time the worker is retarded as shown by the lower nitrogen, lipid and energy content. The queens attained a maximum live weight of approximately 250 mg. during development while the workers averaged 150 mg. The queens reached a maximum nitrogen content of 5.5 mg. per individual and the workers about 2.0 mg. The highest total lipid for the queen caste was 13.0 mg. per individual while the worker was about 5.0 mg. The energy changes accompanying the development of the female castes were studied by determining the total calories per gram of dried tissue. The maximum attained by the queens was 6750 calories per gram of dried tissue and the workers 5690. It appears the either a qualitative or quantitative dietary deficiency is a contributing factor causing the dimorphic condition because each female larvae is potentially capable of developing the characteristics of the queen or worker caste.

105. AN EXPERIMENTAL STUDY OF CHROMATIN DIMINUTION IN *ASCARIS*. R. L. King and H. W. Beams, State University of Iowa. (Lantern; 10 min.)

Early development in *Ascaris equorum* is dependent upon the integration between nuclear division, cytoplasmic cleavage and progressive changes occurring in the cytoplasm. Chromatin diminution depends upon the local elaboration of a specific chemical substance, D, from a cytoplasmic material, An, which is more concentrated at the animal than at the vegetal pole of the uncleaved egg. During cleavage a differential segregation of $An \rightarrow D$ takes place, so that the nuclei of certain cells undergo chromatin diminution, while those of others do not.

Cleavage is suppressed by high centrifugal forces, but nuclear division and the process $A \rightarrow D$ are relatively unaffected. Suppression of cleavage by centrifuging results in multi-nucleate cells; these may divide into the expected number of daughter cells after return to normal conditions. The nuclei of the resulting daughter cells are usually similar in that they have already undergone chromatin diminution or do so at the next division. Suppression of partition walls results in failure of differentiation because the diminisher, D , has diffused throughout the uncleaved egg.

The centrosomes divide, asters and spindles appear, chromosomes are formed and divide, and normal daughter nuclei are reconstituted, while the egg is exposed to high centrifugal forces. The subsequent development of eggs is not affected by long-continued exposure to high centrifugal forces if such are returned to normal conditions before the first cleavage mitosis occurs.

106. THE STRUCTURE OF SALIVARY CHROMOSOMES IN *SIMULIUM*. T. S. Painter and Allen B. Griffen, University of Texas. (Lantern; 15 min.)

The giant salivary chromosomes are made up of four bundles of chromonemata; each bundle originates from a single chromomeric chromosome (chromatid) and contains usually about sixteen strands. The great size of the giant chromosomes is due mostly to the growth of hypertrophy of the individual chromomeres and to some elongation of their connecting threads. The four original chromatids are twisted together early in development and as the chromomeres increase in size they become appressed in the transverse plane, a process which often forces out of place the chromatin which forms a hull about the vesicle of the chromomere. The appearance of any given area therefore depends on the degree of hypertrophy of the chromomeres, a fact which must be considered in interpreting salivary chromosome maps and figures. Few of the 'bands' in *Simulium* are made up of one row or disc of chromomeres, more usually there are two or more closely connected linearly. The chromomeres of the very early prophase probably correspond to general banded areas and not to single bands in the salivary chromosomes.

107. A STUDY OF THE CHROMOSOMES OF A SEX REVERSED FEMALE AND OF NORMAL MALES OF THE COMMON FOWL, *GALLUS DOMESTICUS*. Richard A. Miller, State University of Iowa. (Introduced by C. B. Davenport.) (Lantern; 15 min.)

A Buff Orpington female sinistrally ovariectomized 4 days after hatching developed a testis-like structure at the site of the right gonad. The bird was stimulated with gonadotropic hormones and killed at the age of 11 months. The testis-like structure showed all stages in maturation including spermatozoa. The appearance and behavior of the chromosomes during meiosis are compared in this ovariectomized female and in testis from similarly stimulated males.

The form of the large chromosomes is described in spermatogonial and spermatocyte divisions. The fifth chromosome in order of size, a V-shaped element with median spindle fiber attachment is the sex chromosome. In male spermatogonia the element is paired; in female spermatogonia it is single.

The fifth spermatocyte tetrad of males is a heavy element formed by the pairing of homologous spermatogonial chromosomes. In the female the corresponding element is a slender chromosome resembling a spermatogonial chromosome. It passes without dividing to one pole of the spindle ahead of the other chromosomes. In males the fifth tetrad segregates normally one chromosome moving to each pole.

It may be concluded that sex reversal in the ovariectomized fowl does not involve a change in the chromosome complement.

108. OOGENESIS OF *CAMPPELOMA RUFUM*, A PARTHENOGENETIC SNAIL. Norman T. Mattox, Purdue University. (Introduced by Harley J. Van Cleave.) (10 min.)

The primary oogonia which arise from indifferent epithelium of the ovary average $10\ \mu$ in diameter. After a slight growth period they undergo a division to form the oocytes. During this division, two groups of twelve chromosomes each are distinguished. Based on observations of these cells and somatic mitosis, the diploid number has been established as 12.

Following this division, there is a growth period during which the oocytes reach an average diameter of $35\ \mu$. In the prophase stages no synaptic stages have been observed. Secondary nucleoli develop in the prophase stages to disappear with the formation of chromosomes. A study of these cells had led to the conclusion that only one polar body is formed with no reduction in chromosomal number. The actual polocyte formation has not been observed but in fixed material polar bodies attached to the wall of the oocyte have been seen just after extrusion and in a pyknotic condition. During all divisional stages observed there was a marked tendency for a lagging of chromosomes nearest the center of the spindle.

Following polocyte formation the nucleus of the ovum returns to an interphase and the cell breaks loose from the ovarian wall. While still in the oviduct ova have been observed in the process of cleavage, but only through first cleavage. Apparently further development does not take place until the uterus is reached.

Normal diploid parthenogenesis has been postulated for *Campeloma rufum*.

109. A CYTOLOGICAL STUDY OF THE OVARIES OF THE BATS, *MYOTIS LUCIFUGUS LUCIFUGUS* AND *M. GRISSENCENS*. Mary J. Guthrie and Katherine R. Jeffers, University of Missouri. (Lantern; 12 min.)

Ovaries of bats, *Myotis lucifugus lucifugus* and *M. grisescens*, have been studied in all phases, except gestation, of the annual reproductive cycle. Oocytes arise in the germinal epithelium and grow with an orderly differentiation of the storage products of metabolism—chondriosomes, fat drops and yolk vesicles. The evidence indicates that the zona pellucida is derived from the oocyte. Squamous cells of the primary follicle become cuboidal and then columnar as follicular growth begins (unilaminar secondary stage). The secondary follicle becomes multilaminar and in a very few cases gives rise to a tertiary follicle by antrum formation. Most growing follicles undergo retrogression by one of two methods. Degeneration of type I is common in multilaminar follicles and begins in the granulosa which is almost completely obliterated before the oocyte is affected. Spindles occur in some of these oocytes and fragmentation is common;

phagocytes eliminate the contents of the zona. In type II, which is rare and has been found only in unilaminar secondary follicles, the oocyte degenerates, leaving an uninjured granulosa. Medullary cords are common embryonic vestiges in these ovaries; epithelial nodules are less frequent. Interstitial cells are very numerous in newborn bats, arise throughout life from the hypertrophied thecae internae of retrogressing follicles of type I, are phagocytized and, also, undergo hyaline degeneration, are hypertrophied in late pregnancy, and continue so during lactation.

110. FURTHER OBSERVATIONS ON THE REPRODUCTIVE SYSTEM IN MALE BATS. Roland E. Miller, University of Missouri. (Introduced by Mary J. Guthrie.) (Lantern; 8 min)

A study has been made of the reproductive system in male bats of the species *Myotis grisescens* during the winter months. Previously this species has been available only during the late spring, summer and early fall. Young males of *Myotis grisescens* do not show any spermatogenic activity during the year following their birth. The entire reproductive system remains immature. In adult males the spermatogenic cycle occurs during the summer months at which time the remainder of the system is in an inactive state. Following spermiogenesis and as the spermatozoa are leaving the testis the seminiferous tubules become filled with spermatogonia by mitotic divisions of basal cells. Throughout the winter months the testes are small and inactive. In the late summer, following spermiogenesis, the secretory epithelium of the accessory glands becomes functional, and the lumina remain filled with secretion until spring. The epididymides are found to contain large numbers of spermatozoa from September until the latter part of March when evacuation is conspicuous. Spermatozoa have been found in the lumina of the various accessory glands and in the urinary bladder throughout the winter months. It is possible that the presence of spermatozoa in these regions of the system is correlated with intermittent copulations during the winter.

111. THE GOLGI SUBSTANCE OF INSECT MUSCLE. Herbert L. Eastlick, University of Missouri. (Introduced by W. C. Curtis.) (Lantern; 12 min.)

Crickets, wasps, and grasshopper wing and leg muscles have been studied following fixation in various osmic acid and silver nitrate solutions used for revealing the Golgi substance. In favorable preparations irregular osmiophile or argentiophile bodies occur at the poles of the nuclei or along their sides. This material, because of its typical topography, size, shape, etc., seems to represent the true Golgi substance of insect muscle.

A ramified network of Golgi material has not been noted in this investigation. Since the membranes and bands, and occasionally the tracheoles and walls of the fibrillae reduce silver and osmic acid, a net-like appearance may result from an impregnation of these structures. Evidence in favor of this interpretation has been obtained from fat extracted muscle and from tissue fixed in gold chloride and in iodine vapor.

112. ON THE ULTRASTRUCTURE OF THE BATONNETTES OF HEIDENHAIN IN RENAL TUBULE CELLS OF AMPHIBIA AS REVEALED BY POLARIZED LIGHT STUDIES. Caswell Grave II, Washington University, St. Louis. (Introduced by Francis O. Schmitt.) (Lantern; 10 min.)

Although the physiological roles of the distal convoluted tubule cells in kidney function in Amphibia is coming to be at least partially understood, the cellular and molecular mechanism of these processes is altogether unknown. The most prominent structures in these cells aside from the nuclear are the batonnettes of Heidenhain, which lie well oriented in the cell in a direction perpendicular to the basement membrane. The ultrastructure of these lipoprotein rodlets has been examined by the polarized light method, not only because of the value of such information with regard to their molecular organization, but also with the hope that such an analysis may eventually suggest the possible role of these structures in the physiological function of the tubule cells.

In freshly teased cells or in frozen sections the rodlets show very weak birefringence and tend to disintegrate rapidly. In frozen sections of formalin-fixed material the rodlets are positively birefringent with respect to their long axes. If the section is immersed in aqueous solutions of glycerine (30 to 50%), which have relatively high refractive indexes, the sign of the birefringence is reversed to negative and the possibility of this reversal is abolished by treatment with lipid solvents. After lipid extraction the positive birefringence is largely from birefringence.

These facts suggest that the batonnettes are composed of protein particles or fibrillae extending parallel to the axis of the batonnettes and of lipid molecules oriented perpendicular to this axis.

113. THE NUMERICAL RELATION OF RETINAL GANGLION CELLS TO OPTIC NERVE FIBERS IN VERTEBRATES. Leslie B. Arey, Northwestern University Medical School. (Lantern; 15 min.)

For man Zwannenburg ('15) has estimated the ganglion cells of the retina to total 3,250,000. But this is six times the number of nerve fibers also estimated by him for the optic nerve and about three times the number found by Arey, Bickel and Schaible. If, therefore, it is true that many of the ganglion cells do not send processes back to the brain, then this is a fundamental fact of considerable importance.

To obtain more exact information a study was made on the dog which has relatively few optic nerve fibers of large caliber. Six animals gave a total nerve fiber content ranging from 137,681 to 162,243 (Arey, Castanares and Schaible). A determination of the number of ganglion cells in four retinas from the same animals gave totals of 149,320 to 192,160 (Arey and Gore). Hence there is good reason to believe that a simple 1:1 ratio exists. It is probable that the numerous glial cells of the ganglion cell layer have not previously been excluded from ganglion cell counts.

Similarly, Palmer ('12) estimated the total ganglion cells in the retina of *Necturus* to be 30,000 while six careful counts (Arey and Lander) show the optic nerve fibers of this animal to number 356 to 371. However, it has not been possible to differentiate ganglion cells from the other numerous elements of the ganglion cell layer and thus obtain a differential count.

114. OBSERVATIONS ON GIANT CELLS APPEARING IN TISSUE CULTURES OF AMPHIBIAN SPLEEN. George A. Baitsell, Yale University. (Lantern; 15 min.)

Studies extending over several years on in vitro, hanging drop cultures of amphibian spleen tissue have shown that multinucleated giant cells of extraordinary size and behavior are consistently found in the older preparations. The giant cells usually appear during the second week of 'culture life,' moving slowly out into the clot from some portion of the spleen tissue which is in contact with the cover glass. The cells usually remain attached to the cover glass where their activities may be studied for considerable periods. It is noteworthy that cultures of amphibian tissues do not require frequent transplantation as do the tissues of warm-blooded animals, so that living individual giant cells may be observed for days and even weeks without being disturbed. The size of these cells is quite extraordinary. They commonly measure around 0.4 mm. in diameter when inactive, while cells exhibiting active movement have been observed which measured over 2 mm. in length. They are multinucleate, but with no apparent uniformity in the nuclear number. Observations have frequently shown a budding or fragmentation of the cytoplasm and consequent formation of independent cellular bodies in considerable numbers. On the other hand, the opposite process involving fusion of smaller cellular bodies to form large giant cells is also of common occurrence. The giant cells as described, with accompanying fragmentation and fusion phenomena, are believed to be normal cellular constituents of the spleen tissues of *R. pipiens* and not the result of abnormal culture conditions.

115. MITOTIC ACTIVITY OF STIMULATED RAT ADRENALS AND SPLEEN MEASURED BY COLCHICINE TECHNIQUE. Opal Wolf, Goucher College. (Lantern; 15 min.)

An alkaline extract of the anterior lobe of the pituitary purchased from E. R. Squipp and Sons was injected into rats, one group receiving 1 cc., the other 2 cc. daily. Following the second, fourth, sixth and tenth days of injection 0.3 mg. (per 100 gm. of body weight) of colchicine was injected.

The average count of the three largest sections of the adrenals from control rats was forty-four mitoses. Animals injected 6 days at the 2 cc. level showed greatest stimulation. One male had 232 mitoses, chiefly in the zona fasciculata of the cortex. At the end of 10 days injection, the average count returned approximately to the normal level.

After 2 days on the 2 cc. level the reticular cells in the red pulp of the spleen showed as many as 8 to 10 cells in mitotic activity in one field. After 6 days many centers of lymphoid tissue were noted in the red pulp, the megakaryocytes were more numerous and the follicles were larger. The average ratio of spleen weight to body weight was slightly greater in the injected animals and most marked at the end of 6 days. Following 10 days injection the mitotic activity had decreased and the spleen weight was nearer that of the controls. Many necrotic centers, however, were noted in these spleens.

A comparison of the activity of the adrenal and spleen over the 10-day period furnishes a valuable index of pituitary stimulation and indicates an anti-hormone effect.

116. THE CELLULAR CHANGES INDUCED IN THE TESTES OF THE ALBINO RAT BY ARTIFICIAL CRYPTORCHIDISM AIDED BY THE ARREST OF MITOSES WITH COLCHICINE. Arthur J. Gatz, Carleton College. (Introduced by J. E. Wodsedalek.) (Lantern; 15 min.)

The germinal epithelium of the cryptorchid rat is present in a degenerate condition. After 2 weeks the inner lining of the germinal tubules appears to be a vacuolated fibrous mass. Frequently large deeply stained cells may be seen in the lumen of the tubule. Nine hours after an injection of colchicine mitotic figures may be seen in the above cells. The gross size of the testes is markedly decreased. The germinal tubules have also atrophied to a considerable extent. The decreased size of the tubules causes the appearance of enlarged intertubular spaces which are filled with granular lymphoid accumulations and cords of interstitial cells. The presence of the definite cords of interstitial cells, which appeared to be more numerous than in normal testes, suggests that these cells may be increasing by mitotic division. Mitotic divisions were practically never observed in previous work. The subcutaneous injection of colchicine shows very few, if any, mitotic figures in the interstitial tissues. Measurements of the cytoplasm and the nucleus were also made to determine if changes in cell size were responsible for the seemingly increased amount of interstitial tissue. At present, it appears that the reduction of the tubule size and the gross size of the testis causes a condensation of the interstitial tissue. It appears that there is no hypertrophy of the interstitial tissue.

117. THE STIMULATION OF YEAST GROWTH BY COLCHICINE. Oscar W. Richards, Research Section, Spencer Lens Co. (Lantern; 10 min.)

The budding of yeast is generally believed to be amitotic, although a few investigators present mitotic figures. Colchicine arrests mitosis and aids in the observation of the nuclear material during division. A pure strain of yeast, *Saccharomyces cerevisiae* Hansen, was grown in concentrations of colchicine of 1 part in 1,000,000 to nearly 2 parts in 100 parts of Williams' medium. The drug stimulated yeast at all concentrations used with a maximum at about 1%. The increase depends on the criterion used for the measurement of yeast growth and varies from 142% to 200% of the control with a colchicine concentration of 1%. Colchicine is not a bios as the stimulation is less and much greater concentrations are required than would be expected from a bios. When colchicine is present the population equilibrium marking the end of the first growth cycle is absent and the growth continues to its final level. The drug buffers the medium and may serve as a food. Stained preparations did not show any mitotic figures. The colchicine technique supports the view that the budding of yeast is amitotic.

118. RAM SPERM IN THE GENITAL TRACT OF THE EWE. Virgene Warbritton, Frederick N. Andrews, Victor Berliner, and Fred F. McKenzie, Department of Animal Husbandry, Agriculture Experiment Station, University of Missouri and United States Department of Agriculture, Cooperating. (Lantern; 10 min.)

An attempt has been made to study the effect of the female genital tract on ram sperm. Sperm was found in twelve of sixteen fallopian tubes 2 hours after insemination and were relatively numerous in one tube. Twelve hours after

insemination several sperm were found in four of seven tubes and a few were present in all except one. Twenty-two hours after insemination several sperm were washed from the tubes of one ewe, a few from one, and none from a third. Few, if any more sperm could be washed from the horns of the uterus than from the tubes. Numerous sperm were obtained from most of the cervixes and vaginas. A few sperm were obtained from the tubes of two and horns of three of five pregnant ewes. Sperm were found in the tubes and uteri of ewes inseminated before the beginning of estrus and 1 day after its close but not of the ewe slaughtered during silent heat. The pH of the tubes, horn, cervix, and vagina was determined with a quinhydrone electrode. The abnormality found most frequently is the tailless head. The only other common changes can be referred to agglutination. Leucocytes were rather numerous in some preparations but were very rarely seen attached to the spermatozoa.

119. THE BLOOD VASCULAR SYSTEM OF THE HYPOPHYSIS OF *AMBLYSTOMA TIGRINUM*. Paul G. Roofe, University of Louisville. (Introduced by S. I. Kornhauser.) (Lantern; 10 min.)

The posterior division of the ramus hypothalamicus in supplying all the buccal portions of the hypophysis breaks up into a complex sinusoidal network, the rete of each portion anastomosing freely with the rete of the adjacent portion.

The pars nervosa is supplied with blood from one or two sources, either from the dorsolateral branches of the ramus hypothalamicus or from the superior hypophyseal arteries from the posterior communicating artery.

The entire hypophysis is drained by the hypothalamic veins which enter the rete of the saccus endolymphaticus from below.

The second network of sinusoids found in the rete of the saccus endolymphaticus can in no way be considered a hypophysio-portal system in the sense in which Popa and Fielding ('30, '32, '33) first described such a system. There is no evidence in the material here presented that a second network of capillaries exists in the hypothalamic region in the venous channels from the hypophysis.

120. THE DEVELOPMENT OF THE VALLATE PAPILLA AND ASSOCIATED TASTE BUDS IN THE RAT. Theodore W. Torrey, Indiana University. (10 min.)

The development of the vallate papilla in the rat is instituted toward the end of the eighteenth day of gestation in the form of a circular epithelial down-growth into the underlying connective tissue of the tongue. This epithelial invagination is progressively split by a circular cleft which works its way downward from the surface, thus establishing the trench which bounds the conical papilla. Taste buds do not appear on the walls of the papilla and trench until the ninth day after birth, from which time they gradually increase in number, reaching a maximum in about 12 weeks. The development of associated nerve fibers comes about coincidentally with the appearance of the taste buds. Experimental work in progress is designed to answer the question of cause and effect in this relationship.

121. ON THE SIGNIFICANCE OF THE OCCURRENCE OF MELANIN PIGMENT IN THE CORIUM OF THE SKIN OR IN THE DEEPER PORTIONS OF EPIDERMAL STRUCTURES. R. M. Strong, Loyola University School of Medicine. (Lantern; 15 min.)

Whenever melanin pigment occurs in the form of discrete granules in epidermal structures, brown colors of varying darkness appear, depending upon the amount of pigment present and assuming that there are no other pigments. When melanin pigment occurs in the dermis of the skin and there is no other pigment present, it is commonly present in closely packed masses, usually in so-called melanophores. If in such cases, the epidermis is unpigmented, the skin color has a bluish appearance. In the case of human skin, a pathologic condition is indicated. The phenomena responsible for such conditions will be discussed in the paper.

122. A COMPARISON OF ORGAN-GLAND RELATIONSHIPS IN THE ARCTIC AND THE OHIO MEADOW MOUSE (*MICROTUS DRUMMONDI* AND *MICROTUS PENNSYLVANICUS*). Daniel P. Quiring, Western Reserve University and Cleveland Clinic Foundation. (Introduced by J. Paul Visscher.) (Lantern; 12 min.)

Brain, heart, kidney, lung, adrenal and thyroid gland weights are compared in fifty Ohio and fifty northern *Microtus*. The organ and gland data are evaluated in terms of the relative growth equation. Relative brain and heart sizes appear almost identical in the two species. Body weight is about 12% higher in the local species. Adrenal and thyroid glands, kidneys and lungs are relatively larger in the northern species. The females in both species are larger on the average than the males of their own species. The glands and organs considered in this paper appear larger in the females than in the males. During pregnancy the thyroid appears to be reduced in size.

123. EXPERIMENTAL STUDY OF SURVIVAL VALUE OF ACRIDIAN PROTECTIVE COLORATION. F. B. Isely, Trinity University, Waxahachie, Texas. (Lantern; 15 min.)

Grasshoppers, whose color patterns appear to the human observer to blend perfectly with their natural surroundings, were used for these experiments. The predators, real enemies, were mocking birds, sparrows, cardinals, turkeys and bantams.

A garden plot 12 × 16 feet was marked off into squares 16 × 16 inches arranged in checkerboard fashion to represent four different types of backgrounds: black, white and red soils, the fourth green, transplanted bermuda. The acridians were picketed on the various squares of the checkerboard plot or for some experiments anesthetized. Usually matched pairs of the same species instar and sex were used in equal numbers, i.e., white acridians on white and green squares, black acridians on black, red or white squares, etc.

The native birds could be easily observed and checked from a screened porch and from the house windows. The domesticated birds permitted experimenters to follow their every movement.

The records of thirty-three experiments (June 9 to July 1, 1937) show that out of 459 acridians placed on non-harmonizing or non-protected backgrounds of the checkerboard plot and subjected to predator depredations that 405 or 88.24% were eaten by the birds while 54 or 11.76% survived. On the other hand, of the protectively colored acridians placed on harmonizing backgrounds 276 or 60.11% were missed by the birds while only 183 or 39.85% of the acridians protected by concealing colors were eaten. All experiments show that concealing coloration protects acridians against bird predators.

124. THE LIFE CYCLE OF THE HEN LOUSE *LIPEURUS CAPONIS* (LINN.). F. H. Wilson, Tulane University. (10 min.)

Adult males and females of the biting louse *Lipeurus caponis* (Linn.) were taken from a hen. Each pair, male and female was isolated on a feather in a syracuse watch glass, and placed in an incubator at approximately 33°C. The feather was the only source of food. As eggs were laid the adults were moved to fresh dishes and feathers. The eggs hatched in 4 to 7 days with the great majority of the eggs hatching in 5 to 6 days. The first instar lasted from 6 to 18 days with the majority of the individuals taking 7 to 11 days. The second instar lasted 5 to 16 days, usually 6 to 9 days. The third instar lasted 6 to 9 days at which time the adults emerged. Three adults were reared. The three adults appeared 24 days, 29 days and 32 days, respectively, from the date on which the eggs were laid.

In these experiments, a female laid thirty viable eggs after her male partner had died. The last viable egg was laid 31 days after the death of the male.

125. A METHOD OF DETERMINING THE MEAN SPEED OF MOVEMENT OF *TRIBOLIUM* BEETLES MOVING THROUGH A MASS OF FLOUR. John Stanley, Queens University, Kingston, Ontario. (Introduced by Charles P. Sigerfoos). (10 min.)

Using special equipment a laminated flour mass is laid down in a heavy-walled steel cylinder, the laminae being alternately white and black (the black being produced by the addition of carbon black), and 1 mm. thick.

After a beetle has bored in the flour for a known period, the mass is compressed at approximately 15,000 pounds per square inch, removed from the mould, and sectioned in 1 mm. slices at right angles to the laminae.

The trails can then be seen as gray streaks, and are reconstructed by mapping on sheets of celluloid. From the length of trail per unit time the mean speed of movement can be calculated.

DEMONSTRATION PAPERS

126. REPRODUCTION AMONG MAMMALS. Heluf H. Strandkov, The University of Chicago. (Motion picture.)

A 9-minute motion picture with sound, showing the basic features of reproduction among mammals. The pig is used as the primary source of illustrative material. The film is one of a series of biological motion pictures developed by The University of Chicago in conjunction with the Erpi Picture Consultants of New York City.

127. OVULATION AND EGG FORMATION IN THE HEN. D. C. Warren and R. E. Phillips, Kansas State College. (Motion picture.)

Because of the large size of its ova the domestic hen is very favorable material for the study of ovulation. By operative technique a considerable number of ovulations and passages of the ovum through the oviduct were observed. Some of the observations were recorded in the motion picture film to be presented. Ovulation usually occurs within 30 minutes after the previous oviposition. The ovum is released in the cavity about the ovary and immediately engulfed by the infundibulum. Approximately 3 hours is required for the secretion of the egg white, and $1\frac{1}{4}$ hours for the development of the egg membranes. The egg spends about 20 hours in the shell gland although shell formation seems to be completed in a much shorter period.

128. THE 'RESONANCE' CHARACTER OF NERVOUS CONTROL, DEMONSTRATED BY THE 'HOMOLOGOUS' RESPONSE OF TRANSPLANTED MUSCLES AND LIMBS. Paul Weiss, The University of Chicago. (Motion picture.)

The moving picture illustrates the basic phenomena which have led to the discovery of the resonance-like character of central-peripheral relations in the nervous system.

Basic facts: 1) Each skeletal muscle owns a constitutional (presumably biochemical) personal characteristic distinguishing it from all other muscles. 2) By virtue of this individual specificity each muscle 'tunes in,' as it were, its motor nerves; that is, it renders them specifically receptive for central impulses of a form appropriate to the muscle at the end of the line. 3) The centers, employing various impulse forms corresponding to the variety of peripheral muscles, activate a muscle by discharging its specific 'call'; to this the motor neurones of corresponding specificity respond selectively.

The film demonstrates the following manifestations of this 'resonance' principle: 1) An implanted supernumerary muscle connected with a strange nerve 'retunes' the nerve so as to make it selective for the new muscle. Accordingly, the transplant contracts whenever a central 'call' is given out for the normal muscle of the same name. 2) In a supernumerary transplanted limb each muscle reacts as under point 1; consequently, the transplant duplicates the movements of the normal nearby limb, if both are of the same asymmetry, and mirrors it, if the limbs are of opposite asymmetry (right and left). 3) Interchange of right and left limbs results in peripheral reversal of the central walking pattern: animals intending to advance actually recede. 4) A 'resonance' relation similar to that of the motor field is also responsible for the central identification of sensory messages coming from muscles; specific reflex responses illustrate this point.

129. TRANSPLANTED WINGS AND HIND LIMBS OF CHICK EMBRYOS PREPARED BY THE METHYLENE BLUE METHOD. Molly Waugh and Viktor Hamburger, Washington University, St. Louis.

The primordia were 2 to $2\frac{1}{2}$ days old when transplanted and the hosts were 8 to 15 days old when fixed. The preparations show complete self-differentiation of girdles and of free appendages and normal formation of joints (in absence of functional activity).

See abstract no. 4.

130. THE HORMONES OF THE HYPOPHYSIS OF THE TURKEY. G. M. Riley, A. J. Stanley and E. Witschi, State University of Iowa.

Continued assays of the dry powder from 6000 turkey hypophyses prove the presence of the chromatophorotropic, two or three gonadotropic, the thyrotropic, parathyrotropic, mammatropic and adrenotropic hormones. Whether certain growth effects in tadpoles are due to the growth hormones or represent a general effect of the other hormones in the injected powder is not ascertained. The striking similarity in the hormonal contents of bird and mammalian hypophyses contrasts with the unlikeness of some of the hormone controlled reactions in the two classes. It appears that in the course of evolution effector systems change more than the related inductors. Reactions produced in different animals will be demonstrated.

131. ABNORMALITIES INDUCED BY CENTRIFUGING FROG EGGS. H. W. Beams and R. L. King, State University of Iowa.

Frog eggs have been fragmented in the ultracentrifuge. When centrifuged in the many cell or early gastrula stages, various types of abnormalities may occur. Tadpoles with two tails, abnormal neural tubes and abnormal brains are common. In a low percentage of cases, white tadpoles occur. This condition is apparently due to an abnormal development of the hypophysis.

132. THE EFFECT OF PITUITARY IMPLANTATION ON ADVANCING SEXUAL MATURITY IN *AMEIURUS MELAS*. Waldo Shumway and Arnold L. Soderwall, University of Illinois.

Preliminary experiments on the effect of pituitary glands of *Ameiurus* injected whole into the body cavity of young adults of the same species (four males, two females) showed an appreciable advance in sexual maturity displayed by the experimental animals over the controls. After daily injections for 6 days, the animals were sacrificed on the eighth day. Mature oocytes were observed in the females, and mature sperm in the males, of the experimental series, while the control animals exhibited the reserve oocyte and spermatocyte stages, respectively. It is planned to continue these experiments and to determine whether ovulation can be induced by this procedure.

133. ADAPTATIONS FOR VIVIPARITY IN EMBRYOS AND OVARY OF *ANABLEPS ANABLEPS*. C. L. Turner, Northwestern University.

See abstract no. 11.

134. AXIAL DUPLICATION IN SERPENTS. Bert Cunningham, Duke University.

There have been described in scientific literature some 200 specimens of serpents showing some degree of duplication of the skeleton. These may be divided into those in which the degree of duplication has not been determined and those in which it was determined. The latter group may be divided into those with: a) head part duplicated, b) head and part of the body duplicated, c) tail parts

duplicated, d) both head and tail parts duplicated with the body single. Seventeen of these are described here for the first time, and additional information is given for six previously mentioned but not described specimens. Furthermore all previous figures, insofar as is known, have been collected and these along with the pictures of the newly described species are shown in the lantern slides. Based upon the reported cases of duplicity it would appear that, for the most part, this phenomenon in serpents originates either from multiple embryonic disks upon a single yolk, or multiple organization centers upon a single disk.

135. SOME MAMMALIAN ABNORMALITIES. Albert M. Reese, West Virginia University.

The exhibit consisted of anterior appendages of the domestic cat with seven digits and of the skeleton of an abnormal lamb. The latter was received as a damaged and partly cleaned skeleton, without history. There is a single head, articulating with the atlas vertebrae of two skeletons, fused in the thoracic region but with distinct appendages and posterior parts.

136. THE STRUCTURE OF SALIVARY CHROMOSOMES IN *SIMULIUM*. T. S. Painter and Allen B. Griffen, University of Texas.

See abstract no. 106.

137. AN EXPERIMENTAL STUDY OF CHROMATIN DIMINUTION IN *ASCARIS*. R. L. King and H. W. Beams, State University of Iowa.

See abstract no. 105.

138. DEMONSTRATIONS OF X-Y CHROMOSOMES IN FIRST SPERMATOCYTES OF MAN. R. L. King and H. W. Beams, State University of Iowa.

No abstract received.

139. MULTIPLE CHROMOSOMES OF *PARATYLOTROPIDIA BRUNNERI* (ORTHOPTERA, ACRIDIDAE). R. L. King and H. W. Beams, State University of Iowa.

There are nineteen chromosomes in spermatogonia of which four are V-shaped and fifteen rod-shaped. There are twenty chromosomes in female somatic cells of which four are V-shaped and sixteen rod-shaped. Each of the V-shaped chromosomes appear to be a multiple formed by the union of two rod-shaped chromosomes; the basic number of chromosomes being that found in most other Acrididae (23 male; 24 female). One of the V-shaped chromosomes in the male and two in the female consist of the accessory united with another rod-shaped chromosome.

In the first spermatocyte there are nine chromosomes, seven telomitic tetrads, one large elongated O-shaped octad, and a decad, made of two V's and a rod. One of the V's of the decad includes the accessory chromosome which may be recognized by characteristics usual for Acrididae. The other V of the decad is apparently limited to the male, one of its arms being homologous to the free rod of the decad, the other to the rod fused to the accessory. This condition is unique for this much studied group and is similar to the tripartite sex chromosome of the first spermatocyte of the mantids.

- 139A. NUCLEAR DIVISION IN CHAOS CHAOS LINNAEUS. M. Catherine Hinchey, Temple University. (Introduced by D. H. Wenrich.)

See abstract no. 71.

140. SPERM PRODUCTION IN RAMS IN RELATION TO THE THYROIDS AND PITUITARIES. Victor Berliner and Virgene Warbritton, Agricultural Experiment Station of the University of Missouri and United States Department of Agriculture, cooperating.

Observations on thyroids, pituitaries and testes from fourteen rams have been correlated with the sperm production of these rams. Sperm production was checked for approximately 2 years. Thyroid and testes samples had been taken from several of the rams once, at least. Three were completely thyroidectomized and one was vasectomized several months before slaughter. The thyroids and pituitaries from rams of high sperm production may be described as active glands and those from inferior rams as hypofunctioning or storing glands. The testes from the superior rams were characterized by well-developed seminiferous tubules; and from the inferior rams, by hyperplasia of the interstitial tissue. Bio-assays of half of each pituitary for gonadotropic and thyrotropic hormones were less successful than the histological methods in demonstrating differences but suggested the same types of variation.

Breed differences which appeared in sperm production during the summer were paralleled by differences in the thyroids, but not in the pituitaries, of the rams at the time of slaughter. The reaction of the thyroid to either external or internal stimulation might be regarded as a hereditary constitutional factor of a breed.

141. VISCOSITIES OF TUMOR AND OF NORMAL CELLS AS DETERMINED BY THE ULTRACENTRIFUGE. M. F. Guyer and P. E. Claus, University of Wisconsin.

The great viscosity of most tumor cells, particularly adenofibromas and carcinomas, is indicated by their resistance to displacement of cellular contents when centrifuged at extremely high speeds. Implants of several types of cancer into various normal tissues followed by rotation in the ultracentrifuge of excised bits of united host and graft tissue will be demonstrated to show the greater degree of stratification of cell contents that occurs in normal tissues. Duplications of chromosomes without cytoplasmic division are commonly seen in various kinds of cancer cells after rotation for an hour at a speed which produces a displacement pull of approximately 400,000 times that of gravity.

142. THE VACUOLES AND VACUOLAR ACTIVITIES OF THE MARINE AMOEBA, FLABELLULA MIRA. D. L. Hopkins, Johns Hopkins University.

Mitochondria which stain with Janus green B are not present in normal fully active amoebae, but appear and may be stained with Janus green B when the amoebae are placed under slightly abnormal conditions. The mitochondrial substance in fully active amoebae is contained in very small vacuoles. These vacuoles arise de nova from the protoplasm, grow, fuse with each other and with engulfed

food. The food vacuoles and vacuoles containing no food fuse with each other forming larger vacuoles, until finally a large fusion vacuole, the cloacal vacuole, is formed; the contents of which after a period of digestion are voided to the outside. When for any reason protoplasmic streaming stops these vacuolar activities cease also.

Nile blue sulfate stains all these vacuoles blue, from the time of their origin until after their entrance into the cloacal vacuole where the blue fades out becoming clear of color directly or pink and then clear. This indicates that fatty acids are present in all vacuoles and perhaps some neutral fat, both of which types of substances disappear from the vacuoles before the residue is voided.

Substances stained by neutral red are present in all the small vacuoles but disappear entirely before these vacuoles enter into the cloacal vacuole.

Neutral red staining indicates that the smaller vacuoles and the cloacal vacuoles are more highly oxidized than are the vacuoles of intermediate size, and that the vacuoles are acid in the earlier stages but become distinctly alkaline just before the neutral red disappears from them.

143. THE USUAL MORTALITY THAT CHARACTERIZES A PARAMECIUM CULTURE. Edgar P. Jones, University of Akron.

See abstract no. 13.

144. INTRACELLULAR PARASITISM IN FISH PRODUCING A GIGANTIC GROWTH OF THE INFECTED CELLS. Richard Weissenberg, Washington University, St. Louis. (Introduced by Roscoe B. Hyde.)

See abstract no. 75.

145. SEVERAL SUBSTITUTES FOR THE STANDARD MICROTOME KNIFE. Robert T. Hance, University of Pittsburgh.

The high cost as well as the difficulty experienced by many in keeping a standard microtome knife sharp has led the writer to search for substitute blades that are manufactured for other purposes. Several types of blades have been found that are available in most 10 cent and hardware stores. Their cost ranges from 5 cents to 65 cents.

146. A MICROTOME THAT STUDENTS CAN MAKE. Robert T. Hance, University of Pittsburgh.

A microtome easily constructed of wood and of standard metal parts can be put together at a cost of less than 50 cents. The sections produced are not of constant thickness but good and thin sections can be selected for mounting. It is believed that this device may make micrology available as a hobby course in the secondary schools.

147. A MECHANICAL DEMONSTRATION OF THE EFFECTS AND INTERRELATIONSHIPS OF THE HUMAN FEMALE HORMONES OF REPRODUCTION. Wayne L. Whitaker and R. Dean Schick. University of Michigan. (Introduced by Alvalyn E. Woodward.)

This hormone demonstrator combines the pictorial scope and accuracy of a graphic chart with the dynamic, physiological changes of a living organism. A series of appropriately colored liquids rise and fall in glass tubes at varying rates and in regular sequence to illustrate the activity of the several glands of internal secretion which influence the human female reproductive cycles. The demonstrator makes clear the sequence in which these hormones are effective, their interplay, and their effects upon the ovary, influencing the time of ovulation. It also clarifies their effects upon the endometrium and breasts, preparatory to pregnancy or menstrual degeneration of the uterine lining. The source of each hormone is explained with notes on its chemical and physical nature, tests, etc. Printed on a revolving drum and correlated with the animated graphic fluids, is a day by day explanation of interrelationships which are taking place as the observer follows the normal menstrual cycle. Another drum explains hormonal and embryological changes occurring during the pregnancy cycle, which the observer may induce at will (within indicated limitations of fertilization) by moving a lever. Illustrations depict the development of an ovarian follicle and the course of the fertilized ovum, changes which occur in the uterine lining in response to endocrine stimulation for reception of the egg, and changes in form and size of the fetus. A large colored-line graph summarizes the complete menstrual-pregnancy cycle, facilitating interpretation of the animated columns.

148. EXPERIMENTAL PRODUCTION OF EXOPHTHALMOS COMPARABLE TO THAT FOUND CLINICALLY. George K. Smelser, Columbia University.

Heretofore experimentally produced exophthalmos has depended upon sympathetic excitation and contraction of unstriated orbital muscles. It seems quite certain that this mechanism is not the one responsible for the severe exophthalmos of exophthalmic goiter, particularly the 'malignant' or 'progressive' type which persists after thyroidectomy and subsidence of other manifestations of thyrotoxicosis.

Injection of a partially purified anterior pituitary extract into thyroidectomized guinea pigs produces a definite and in some instances an extreme exophthalmos. The protrusion is due to an increase in the mass of retrobulbar tissue, and is readily demonstrable postmortem or in roentgenographs. Ablation of the cervical sympathetic ganglion has no effect on these reactions although the exophthalmos is apparently less. At autopsy the mass of retrobulbar tissue is found to be increased and appears oedematous. One lacrimal, the extra-ocular muscles, and orbital fat and connective tissue are involved but not a second and more ventral lacrimal. Histological examination reveals an oedematous infiltration of these structures which is most marked in the fat and connective tissue and is accompanied by round cell infiltration.

Clinical exophthalmos is caused by an increase in retrobulbar fat, connective tissue and muscles. These tissues are markedly modified by an oedematous in-

filtration which is accompanied by round cells of various types. This condition is demonstrable in both hyperthyroid and in 'progressive' post-thyroidectomy exophthalmos.

The experiments reported here closely reproduce the essential features of clinical exophthalmos.

149. EFFECTS OF THEELIN UPON THE REPRODUCTIVE SYSTEM OF MALE ANOLIS CAROLINENSIS. M. L. Clapp, Montana State University. (Introduced by L. T. Evans.)

During December, 1935, from $4\frac{1}{2}$ to $11\frac{1}{2}$ r.u. of aqueous theelin (Parke Davis) was administered to each of sixteen young male lizards (average weight, 2.3 gm.). When compared with eleven young males retained as controls and killed the same week, the injected animals showed the following histological changes in the reproductive structures:

1. The testes were 40% of the weight of those of controls. The width of the seminal tubules was 69% of those of controls. Whereas controls showed relatively little cellular debris in the lumen of the tubules and the germinal layer was usually six to eight cells in thickness with spermatids and spermatozoa present in small numbers, injected tubules showed much luminal debris, a germinal epithelium usually four cells or less in thickness, and exceedingly few secondary cytes, spermatids or spermatozoa.

2. The diameter of the treated vas deferentia averaged 12% greater, with a smaller lumen, than that of controls. The height of individual cells of the vas of controls averaged 60% of those of injected males although in cross section the control vas averaged twenty-nine cells while the injected averaged twenty cells.

3. The cloacae were 9% larger in diameter and their epithelial linings were more highly folded than those of controls.

4. No changes were detected in the hemipenes.

150. SEX REVERSAL IN THE FIGHTING FISH, BETTA SPLENDENS. G. K. Noble and K. F. Kumpf, American Museum of Natural History.

Spayed *Betta splendens* can regenerate a gonad from the cut end of the oviduct but this will be a testis and not an ovary. Such a regeneration occurred seven times in 150 spayings. Three months after the operation the fish began to develop typical male fins. Three sacrificed at this time showed active spermatogenesis and some mature sperm. Four fish retained 149 to 163 days after the operation showed full development of the male fins. When placed with normal females three spawned normally and produced fertile eggs. The fourth displayed typical male behavior during nine successive spawnings but failed to fertilize the eggs. Serial sections of the gonads of the first three fish showed no ovarian tissue but only testicular tissue undergoing active spermatogenesis. Sections of the gonad of the last showed an organ essentially like a testis but lacking spermatogenesis.

Young reared from breeding the transformed females with normal females were found to be of both sexes. At the present time nine males and twelve females resulting from these spawnings have reached maturity.

Castrated males regenerate their gonads more frequently than do spayed females. The regenerated organ is invariably a testis.

151. THE HORMONAL FUNCTION OF THE CORPORA ALLATA IN THE GRASSHOPPER, *MELANOPLUS DIFFERENTIALIS*. Isabelle G. Weed, State University of Iowa. (Introduced H. W. Beams.)

See abstract no. 103.

152. A NEW METHOD FOR SIMULTANEOUSLY RECORDING THE ELECTRICAL AND MECHANICAL CHANGES IN THE INSECT HEART. Frederick Crescitelli and Theodore L. Jahn, State University of Iowa.

In connection with studies on the physiology of the grasshopper heart a new method has been utilized which permits the simultaneous recording of both the electrical and mechanical changes which occur during the cardiac cycle. The apparatus, whose construction and operation will be demonstrated, consists of a very light glass-silver mechano-electrograph which is essentially a silver-silver chloride electrode modified so as to be capable of registering the heart beat. The cardiac electrical variations, after suitable amplification, are recorded by photographing the beam of a cathode ray oscillograph on the emulsion side of a moving strip of sensitized paper. The mechanical changes are simultaneously recorded through the reverse side of the paper by photographing the shadow of a hair attached to the mechanograph. The use of a microscope makes it possible to obtain suitable amplification of the cardiac movements.

The method is of advantage because of its simplicity, its sensitivity and because it may be applied without fear of producing injurious or disturbing changes in the tissue under examination. It has been shown, by using the frog heart as experimental material, that no electrical artefacts are introduced by using the electrode as a mechanograph.

Records will be exhibited which demonstrate the relationship of the electrocardiogram to the mechanocardiogram, the effects of the application of cold physiological solution, of adrenalin, and of ionically unbalanced solutions.

153. A NEW KYMOGRAPHIC INK-RECORDING PEN FOR THE PHYSIOLOGICAL LABORATORY. T. L. Patterson, Wayne University.

The pen is made from the calamus or quill of a large stiff feather obtained from the wing or tail of an adult chicken (*Gallus domesticus*). The end attached to the fowl is tapered to a point with a thin, flexible razor blade, care being taken to preserve the firm pith in the end of the quill which acts as a plug to keep the ink from flying out of the ink pocket. A flexible shaft electric or dental drill of not over $\frac{1}{32}$ inch is used for making an opening in the quill just above the firm pith at the tapered end and the corner of a razor blade is then introduced into this opening and drawn downward, splitting the quill as nearly as possible through the center of the pen point and trimming. Time is saved if all these steps are carried out under a binocular microscope. Next, an opening is cut on the upper side of the quill, just in front of where the solid, white pith begins and the loose scales of pith below are removed by means of a small wire with a short hook on the end. This forms an ink pocket where a special kymograph ink is introduced.

The pen may be used satisfactorily on all types of recording instruments (manometers, tambours, levels, signal magnets, tuning forks, etc.). It is important that a heavy grade of kymograph paper be employed. Photographic reproduction is also possible.

154. EVOLUTION OF A GASTROPOD SPECIES. H. B. Stenzel and R. W. Cumley, University of Texas.

A biometric study of the first whorls of *Turricula gabbi* Conrad demonstrates that certain shell structures undergo a gradual change through succeeding generations of this gastropod. The number of crescentic ribs on the first five whorls of the shell decreases gradually and without reversals from 188.5 (average) and 191.2 (median), in the earliest known populations, 85.0 (average) and 83.0 (median) in the last known population of the species. Similar changes are recorded in other characteristics of the shell. A large number of succeeding generations are available in the fossil populations of *Turricula* which are found in nearly 500 of marine deposits in Texas.

155. THE SNAKES OF THE UNITED STATES AND CANADA. A. H. Wright and A. A. Wright, Cornell University.

In our endeavor to photograph alive and color describe the reptiles of the United States, we have mounted plates of 138 species and subspecies of snakes east of the Rockies. Any one who could loan us live specimens of *Drymobius margaritiferus*, *Ficimia cana*, *Lampropeltis alterna*, *Lampropeltis elapsoides virginiana*, *Leptodeirs septentrionalis*, *Micrurus barbouri*, *Pituophis ruthveni*, *Pituophis deppei deppei*, *Trimorphodon wilkinsonii*, *Diadophis regalis regalis* will help to supply the missing plates. We have also illustrated the debatable forms. The species and subspecies west of the Rockies are not yet mounted and of these we need about fifteen forms: *Chilomeniscus* (any species); *Crotalus* (*abyssus*, *nuntius*, *stephensi*); *Diadophis* (*occidentalis*, *pulchellus*, *vandenburghii*); *Lampropeltis* (*conjuncta*, *yumensis*); *Leptotyphlops* (*cahuilae*); *Tantilla* (*wilcoxi*); and *Thamnophis* (*biscutatus*, and new species of 1936 and 1937).

PAPERS READ BY TITLE

156. CUMULATIVE GROWTH AND TIME. Otto Glaser, Amherst College.

Whatever the criteria of growth, timing must begin when growing begins. A convenient zero-hour is fertilization; in chicks, 'incubation time' yields consistent results because time 'lost' between laying and incubation is irrelevant. Comparison with mammals may require an addition to the chick time scale.

We must also know what our criteria mean. In the category, fresh weight, water is quantitatively dominant. Quantitatively organized complexes such as chlorides or antlers, or fats with few and relatively undifferentiated molecular species, behave like units. The basic units of course are specific chemical entities, such as water, glycogen, purine nitrogen, etc. In a succession of time units, every 'pure' category increases by a diminishing percentage of itself. 'Interest rates' fall inversely proportional to the differences in the square of time. Integration yields, $\log w = k \log (2t + 1) + C$, with C the integration constant and k the growth constant.

Intervals with k constant are called stanzas. These are limited in duration and not identifiable with growth 'cycles.' Properly employed, stanzas and stanzaic discontinuities enable us to calculate the average weight of cows with an error of less than 1%; to account item by item for the upward swing of dry substance in the chick embryo; and to determine the amount of Ca absorbed by the chick from the shell.

157. THE NUMERICAL VALUE OF THE HETEROGONIC CONSTANT. Otto Glaser, Amherst College.

Rough statistical analysis of 275 examples gleaned from the literature indicates that heterogonic constants fluctuate about unity. What does this mean?

Organic quantities in heterogonic correlation are the immediate or indirect consequences of growth. Whenever our measurements stand for specifiable entities, a stanza of cumulative growth in weights x and y is described by $\log x = k_x \log (2t + 1) + C_x$ and $\log y = k_y \log (2t + 1) + C_y$. From this it can be shown that the heterogonic constant is the ratio k_x/k_y or k_y/k_x .

Twenty serialized growth constants representing categories or entities of the chick embryo range from 6.34 to 3.53. Each constant reflects the sum of the transactions concerned in the accumulation of its respective category. Frequently a given rate differs from its immediate neighbors by less than 5%. In addition there are several groupings of two or three parallel rates designated as isogonic diads, triads, etc. Most significant of these are the heart-water-chloride triad with rates, respectively, 3.59, 3.58 and 3.53; and the fresh weight-purine nitrogen diad with 3.76 and 3.77. All the ratios possible among growth constants with these properties, necessarily fluctuate about unity. This means that organisms are really organized, and during differentiation maintain the compatibilities because certain basic rates are parallel.

158. THE GEOMETRICAL BASIS FOR HETEROGONIC CORRELATION. Otto Glaser and George P. Child, Amherst College.

X and y in $y = bx^k$ are weights or lengths at anatomical levels, or weights of chemical complexes or specific entities. Since the equation, in general, works fairly well, all these measurements must be proportional to some fundamental quantities. We approach the problem at the cytological level. Observations on cellular and nuclear inclusions enable us to postulate hexoctahedra below the limits of visibility.

Stacked in n layers about a central unit, all individuals, S_n , present in isogonic aggregates total $S_n + n - 0.5 = \frac{(2n+1)^3}{2}$. Essentially, $\log S_n = 3 \log (2n + 1) - 0.3010$. This statement is identical in form with $\log w = k \log (2t + 1) + C$, with weight equated against time.

Correlates x and y are produced by growth. If accumulation in x and y is proportional to, or ultimately controlled by, isogonic hexoctahedral aggregation, layer number in such aggregates is proportional to time. Accordingly S_n should be able to replace either x or y in $y = bx^k$. This is true and the resulting 'heterogonic k ' equals one-third the growth constant of y if x is replaced or vice versa.

While these proportionalities fail to identify layer number with the number attached to a specific time unit, they do explain why no workable sample of an organism is out of adjustment with any other workable sample of the same organism.

159. CHEMICAL NATURE OF AMPHIBIAN ORGANIZER. III. CHEMICAL STIMULATION OF THE PRESUMPTIVE EPIDERMIS. L. G. Barth, Columbia University.

Experiments using cephalin showed that preparations that induced neural tubes were toxic and caused cytolysis. Other cytolytic agents were tried and it was found that digitonin in concentrations of 0.05% to 1% in egg albumen stimulate the formation of neural plates in *Amblystoma mexicanum*. Egg albumen buffered at pH 3.0 caused similar reactions in the ectoderm.

With regard to the naturally occurring organizer a comparison of the ether-alcohol soluble fraction and the protein residue of both calves' brains and frog gastrula showed that the protein residue gives better inductions. It is suggested that the ether soluble fraction stimulates by partial cytolysis of the cells while the protein residue may contain the naturally occurring organizer.

160. FORE LIMB OPERCULAR PERFORATION IN *R. TEMPORARIA* AND *B. BUFO*. O. M. Helff, New York University.

The histolysis of anuran operculum in fore limb perforation formation has been accredited to the action of autolyzing gill tissue, to cutaneous gland secretions, and to self-degeneration of the operculum by Helff, Weber, and Blacher, respectively. The writer has reinvestigated the problem as follows.

The right fore limbs (in early stages of development) of *Rana temporaria* and *Bufo bufo* tadpoles were extirpated. Normal perforations occurred during metamorphosis. Cases of double perforations developed, one filled with gill and the other with shoulder girdle.

The right fore limb plus a portion of the shoulder girdle was extirpated in the same species. Normal perforations occurred during metamorphosis. Serial sections gave no evidence of a single cutaneous gland persisting in the peribranchial cavity.

The following conclusions are reached: 1) Limb and shoulder girdle cutaneous glands and gill tissue exercise histolytic effects on the operculum. Gill tissue also functions to enlarge perforations, anteriorly, following fore limb emergence. 2) Following limb extirpation, the gills function more efficiently, histolytically, since the operculum is no longer held away from these organs. 3) Reciprocal skin transplantations in *Rana temporaria* indicate that Blacher may be partially right in that a slight integumentary predisposition may be inherent. 4) The results indicate that fore limb perforation formation is not always the result of the same combination of factors. Perforation formation is 'doubly assured' in some species, and possibly 'triply assured' in others.

161. THE RELATION OF THE DORSAL NERVE CORD AND NOTOCHORD TO TAIL REGENERATION IN ANURAN LARVAE. O. M. Helff, New York University.

Segments of the nerve cord and notochord were extirpated just anterior to the cut-off tip of the tail, from middle regions and from near the junction of body and tail. Tail-tip regeneration occurred normally in all cases of nerve cord extirpation, thereby confirming the work of Goldforb. Removal of notochord from regions just anterior to the cut-off tail-tip results in decided malformation of the regenerated tip. Removal from middle regions and from near junction with the

body results in normal tip regeneration, although usually at a slower rate. Removal from junction with body causes tail to bend at that level similar to cases encountered in artificial metamorphosis. This suggests that the latter phenomenon may be due to structural reactions of notochord to metamorphic agents. When notochord is removed from entire tail, the latter shrinks to a much shortened twisted mass of tissue.

Artificial splitting of the notochord just anterior to the cut-off tip resulted, in several instances, in double tip formation, each containing notochord.

Transplantation of notochordal segments into the dorsal and ventral finny portions results in extension of the fin border distal to transplants, and in some cases, to cylindrical outgrowths of tissue containing notochord, from the distal region of the transplants. Such notochordal transplantation appears to decrease the normal regenerative rate at the tip of the tail.

162. THE INDUCTION OF PIGMENT SPOTS IN *STYELA PARTITA*. S. Meryl Rose, Columbia University. (Introduced by Lester G. Barth.)

In the normal development of *Styela partita* two pigment spots form in the brain from cells which are descendants of the anterior micromeres. When in the 8-cell stage the four micromeres and the four macromeres are isolated, the pigment spots do not develop in either of the isolated halves. There must, then, be some dependence of pigment spot differentiation on the presence of macromere material. This material necessary for the induction of pigment spots in the micromere descendants is located in or develops from the anterior or gray macromeres as shown by the following transplantations: Pigment spots form in the embryos which result from combinations of one or two gray macromeres of the 8-cell stage with all four micromeres or with the two anterior micromeres or with the two posterior micromeres. In this last type of combination the pigment spots are induced in cells which were presumptive ectoderm not destined to form neural cells.

In embryos developing from the two anterior blastomeres of the 4-cell stage or the four anterior blastomeres of the 8-cell stage it is common to find more than two pigment spots, sometimes as many as seven. A part of the explanation may be that the inductor is split up during gastrulation in these partial embryos.

Since pigment spots arise during the differentiation of brain tissue, it may be possible to use them as indicators of a certain stage in neural differentiation. If such is the case, the transplantations show that neural differentiation in *Styela* to the pigment spot stage is dependent upon the presence of material from the anterior vegetal region of the egg.

163. INDUCTION OF DEVELOPMENTAL ACCELERATION BY DEPRESSANTS. J. Wm. Buchanan, Northwestern University.

Single batches of *Amblystoma punctatum* embryos were divided into two lots. One was maintained at 21°, the other was subjected to 6° for periods up to 6 days, then removed to the 21° bath. In every case the development of the cold treated lot accelerated after removal to 21°. Lots started at stage 8 (Harrison scale) were in stage 20 in the 21° bath and in stage 11 in the 6° bath at the end of

72 hours. On the ninth day the control was in stage 41 and the cold treated lot in stage 39. Usually the cold treated lot does not attain complete equality with the control up to stage 45 to 46. Average lengths of the two lots are equal at stage 45 to 46. Inhibition of development by KCN was followed on removal to water by a similar acceleration. Thus following inhibition of development, two lots of embryos of the same genetic constitution under identical environmental conditions develop at different rates. When 2,4-dinitrophenol was applied in attempts to compensate for inhibition at 6° it failed to accelerate development; in concentrations up to 1/10,000% DNP was very toxic, more so at 21° than at 6°. Experiments now under way are designed to examine the respiratory, water metabolism, cytological, and other causes or concomitants of developmental depression and acceleration.

164. OBSERVATIONS OF THE EARLY DEVELOPMENT OF THE ZEBRA FISH, *BRACHYDANIO RERIO*. Eduard Roosen-Runge, Brown University. (Introduced by J. W. Wilson.)

The protoplasm of the zebra fish egg undergoes conspicuous visible changes during fertilization and karyogamy, as well as during cleavage. A motion picture film (1 picture every 1.5 seconds taken by time accelerator) was used to study the separation of protoplasm from yolk and the change in shape of the cells. Exact measurements of single film pictures were made.

Streaming of the protoplasm indicates that surface tension plays a part in the process of bipolar differentiation. As the protoplasm streams from the yolk to form the blastodisc, a counterstream, such as has been postulated in surface tension theories, is clearly visible in the running film. Changes in the shape of the cells as well as of the refraction of granules and alteration of streaming rate demonstrate a pronounced periodicity in the physical state of the egg during the first cleavages.

The protoplasmic changes are striking, due in part to the extreme velocity of development. The time from fertilization to the first mitosis is about 15 minutes at 27°C. (as compared with 15 hours in the trout at 12°C.)

165. A PRELIMINARY STUDY OF SEX DEVELOPMENT IN TURTLE EMBRYOS FOLLOWING ADMINISTRATION OF TESTOSTERONE. Paul L. Risley, State University of Iowa.

Injections of testosterone propionate (13.3 SBU) in the albumen of eggs *Chrysemys bellii* were made in early gastrulation stages. Of thirty-three injected, seven developed normally and were compared with controls that had been injected with the same amount (0.05 cc.) of distilled water. Of the seven experimental animals, two were hypogenitals with strongly reduced gonads, one was definitely male, two were amphisexual with strong medullary activity, and two were females with stronger medullary development than in normal females of similar ages. Secondary sex characters were not significantly altered by the low dosage given. A masculinizing influence of testosterone propionate is clearly indicated in the hypertrophy of the medullary tissues of the gonads.

166. HISTOLOGICAL EVIDENCE INDICATIVE OF THE NATURAL OCCURRENCE OF VITAMIN E DEFICIENCY IN CHICK EMBRYOS. F. B. Adamstone, University of Illinois.

In routine laboratory work a small number of chicks from the eggs of supposedly normal hens develop hemorrhages when removed from the egg in spite of careful handling. Microscopic study of twenty-five such embryos showed conditions apparently identical to those occurring in vitamin E deficient chicks. In the latter the site of rupture of the blood vessel was marked by the presence of rosette-like clusters of characteristic, densely-staining cells with pycnotic nuclei. In the present material the site of hemorrhage varied greatly but was located in twenty-one specimens, twenty of which showed clusters of cells believed identical to those seen in E deficient embryos. Three other specimens also showed the diagnostic cells although the rupture was not located.

Since no other cause of such a condition has yet been described and since it occurs at a time corresponding to the early maximum in embryonic chick mortality, it is suggested that the early mortality peak may be due in part to vitamin E deficiency. The percentage of chicks affected is very small and probably does not exceed 3%.

167. THE RESPONSE OF THE WINGS OF DEPILLATE/WILD, DEPILLATE/VESTIGIAL, AND DEPILLATE/PENNANT OF *DROSOPHILA MELANOGASTER* TO TEMPERATURE. Morris H. Harnly, Washington Square College, New York University.

Depillate is a deficiency covering the region of the vestigial locus. The length and area of the wings of Depillate/Wild vary inversely with the temperature. The curves for these values are nearly identical with those previously reported for Wild/Wild and Wild/vestigial by Stanley, and with those I have reported for pennant/pennant (a vestigial allele). The phenotype changes only in the decreasing frequency of minor nicks and notches between 16° and 32°C. The length and area of the wings of Depillate/vestigial vary directly but not proportionately with the temperature. This genotype does not show the critical temperatures previously reported for vestigial/vestigial. The phenotype ranges from 'apterous' to 'vestigial' with the rise in temperature. The length and area of the wings of Depillate/pennant flies vary inversely but not proportionately with the temperature. The curves have lower values but resemble those for vestigial/pennant between 16° and 22°. Beyond that point the curves differ markedly for the two genotypes. There were no critical temperatures for the Depillate/pennant genotype. Its phenotype varies from 'strap' at 16° to small 'strap' to large 'vestigial' at the higher temperatures.

These three genotypes have been interbred with the vestigial, dimorphous vestigial, and pennant stocks used in my previous temperature experiments. The results for the genotypes employed may be correlated, and general conclusions drawn on the action of alleles at the vestigial locus.

168. THE HISTOLOGICAL STUDY OF THE DEVELOPMENT OF THE COMPOUND EYE IN *DROSOPHILA MELANOGASTER*. Sibyl A. Hausman, Connecticut College. (Introduced by Pauline H. Dederer.)

Recent studies in the development of the compound eye in *Drosophila* have revealed a fact long known, that of a close association with the development of the brain. Although both are of ectodermal origin, they apparently do not begin development together. In larvae but a few hours old (6 to 8 hours) definite bilaterally symmetrical lobes are distinguishable which have been previously recognized as Weismann's 'imaginal discs.' Some 40 hours later these lobes are differentiated into two regions, an anterior 'antennal lobe' and a posterior, concave 'optic lobe.' Around the thickened rim of the concavity are regularly arranged cells which in 48 hours more can be recognized as very rudimentary ommatidia of the eye. The brain at this time is a definite lobulated structure joined by a stalk-like connection to the optic cup.

Observations thus far completed indicate that the elements of the compound eye can be distinguished very early in larval life, before any connection with the brain is established. Eye color can be determined in the 3-day-old pupa. The ommatidia are fully completed on the eighth day. The entire development is a remarkable one, covering less than 9 days. On the ninth, the adults emerge with fully completed eyes.

Material fixed in fluids containing formalin has proved most satisfactory for a histological study of nervous tissues. Celloidin-paraffin embedding, with sections cut at 7μ , results in more clear-cut sections than with the regular paraffin method.

169. THE CHANGES IN THE CENTRAL NERVOUS SYSTEM DURING THE LIFE HISTORY OF THE BEETLE, *PASSALUS CORNUTUS* FABRICIUS. I. E. Gray and Frances Perle Cody, Duke University.

In early embryonic stages of *Passalus* there are, in addition to the brain and sub-oesophageal ganglion, three thoracic and ten abdominal ganglia; one ganglion to each body segment. Before hatching the tenth, ninth and eighth abdominal ganglia coalesce. During the three larval instars the last ganglion remains in the sixth abdominal segment, and only minor changes occur. By the end of the third day of pupal life the adult form of the nervous system is practically assumed. All abdominal ganglia are fused into a single, solid, elongated ganglionic mass. Connectives have disappeared between meso- and metathoracic and between metathoracic and abdominal ganglia; and with the exception of the brain, sub-oesophageal and prothoracic ganglia, the entire ventral nerve chain has come to lie in the mesothorax. The peripheral nerves still arise from the ganglia and ganglionic mass in their same relative positions and still supply the same segments in which they were originally located.

170. THE OSSIFICATION OF THE CHICK SKELETON. II. THE SKULL. Carl J. Sandstrom and Herbert H. Pomerance, University College, New York University.

As a part of a comprehensive study of the development of the chick skeleton by means of in toto and sectioned preparations, the time of the appearance of the centers of ossification of the skull has been determined. The first centers appeared simultaneously at 8 days of incubation in the articulare (first center),

angular, nasal and quadratojugal. Other centers appeared in the following sequence (in days of incubation): $8\frac{1}{2}$, surangular, squamosal, and pterygoid; 9, splenial, dentary (first center), lacrymal, maxilla, quadrate, jugal, and palatine (first and second centers); $9\frac{1}{2}$, frontal, premaxilla (first center), dentary (second and third centers); 10, exoccipital, premaxilla (second center) and ceratobranchial; 11, basitemporal and articulare (second center); $11\frac{1}{2}$, basioccipital; 12, supraoccipital (first center), and parietal; $12\frac{1}{2}$, alisphenoid (first center); 13, postfrontal, alisphenoid (second center), and vomer; and 14, ethmoid, and supraoccipital (second center). Although these observations are in a general way in agreement with those of earlier workers, they serve to record the time of appearance of the centers more exactly.

171. THE OSSIFICATION OF THE CHICK SKELETON. III. THE STERNUM. Carl J. Sandstrom and David F. Sunkin. University College, New York University.

The ossification of the sternum was studied in a series of embryos (stained and cleared in toto preparations) consisting of half-day stages ranging from 7 days' incubation to 1 week post-hatching.

The time of appearance and growth of the primary centers of ossification were variable in contrast to the regularity observed in the skull and appendages. The primary centers of the carina appeared at $16\frac{1}{2}$ to $18\frac{1}{2}$ days (of incubation), one on either side at the anterolateral edge of the concave dorsal cartilaginous plate, and one on either side at the base of the carinal eminence. Fusion of the plates first occurred in the anterolateral region at $18\frac{1}{2}$ to $19\frac{1}{2}$ days. The anterior lateral process ossified from one primary center which appeared at 15 to 16 days. It gave rise to a ring of bone which posteriorly expanded to form two thin parallel plates, one dorsal and one ventral. These plates fused to one another at their lateral and medial edges but remained unfused posteriorly where they would ultimately fuse with the carina. The xiphisternal process ossified from one center which appeared at $12\frac{1}{2}$ to 15 days and which extended for several millimeters along the posterior part of the cartilage of the internal posterior lateral process. This center elongated until it reached the level of the external posterior lateral process where it apparently initiated ossification in that process. Ossification then proceeded anteriorly and posteriorly in the xiphisternum.

172. MEASUREMENT OF THE SPECIFIC CONDUCTANCE OF THE EXTRA-EMBRYONIC FLUIDS OF THE CHICK. Paul A. Walker, Connecticut State College.

The data of previous workers on the growth of the chick embryo have been reviewed and compared with those obtained in the present investigation.

A relationship between weight and length has been devised and employed as an index of the normality of the experimental material.

The specific conductance of the extra-embryonic fluids of the chicks has been measured over the period of development between the seventh and nineteenth days of incubation.

For the allantoic fluid, it is shown that there is an initial 7-day value of 116×10^{-4} mhos/cm., decreasing to about 99×10^{-4} on the eighth day; from this stage to about the sixteenth day there occurs a gradual rise to values of about 105×10^{-4} mhos/cm., and this is followed by a sharp fall to the neighborhood of 76×10^{-4} mhos/cm. on the nineteenth day.

In the case of the amniotic fluid, the conductance values are definitely higher than those of the allantoic fluid up to the fourteenth day, remaining rather constant at about 132×10^{-4} , with a slight maximum on the eleventh day at 136×10^{-4} mhos/cm. Starting on about the fourteenth day there is a marked fall to values of about 81×10^{-4} mhos/cm. on the fifteenth day. This pronounced decrease is followed by a rise to 19-day values of about 122×10^{-4} mhos/cm.

The significance of these data are discussed in relation to previous work on conductance and on the physical and chemical properties of these fluids.

173. THE EFFECT OF OVUM AGE AT TIME OF FERTILIZATION UPON DEVELOPMENT DURING THE PRE-IMPLANTATION PERIOD IN THE GUINEA PIG. Richard J. Blandau and William C. Young, Brown and Yale Universities.

When the effect of ovum age upon the course of gestation was first reported (Am. Soc. Zool., 1936), it was not known whether the structural defects leading to death of the embryo and abortion were attributable to some age-injury of the ovum or to a delay in the time of implantation although the former alternative was favored.

A relatively simple procedure has enabled us to confirm this opinion and learn something about the structural abnormalities found in such embryos during the pre-implantation period. Embryos which developed from ova fertilized approximately 14 hours after ovulation were flushed out of the uterus on the sixth day which under normal conditions is shortly prior to implantation. Since all were abnormal in one way or another and no influence of implantation could have begun to operate, the abnormalities must have been a consequence of the condition of the ovum when fertilization occurred.

When compared with normal 6-day embryos in which the inner cell mass and trophoblast are clearly visible, retarded development, fusion of blastomeres and cytolysis were seen.

174. HOMOTRANSPLANTATION OF THE EMBRYONIC MAMMALIAN DIGESTIVE TRACT. A. J. Waterman, Williams College.

In comparison with the liver, pancreas and lung, the embryonic digestive tract of the rabbit exhibits even more marked survival, regulative and differential capacity in homotransplants within the adult animal. This has been studied in omental grafts of the isolated rudiment and of parts of the youngest embryos from the eighth day of embryonic life to the time of birth. Regional differentiation occurs and is maintained, and the formed tube develops its specific layers. Grafted material is always well incorporated and is in the form of a closed, spherical or elongated cyst with wide lumen except in the case of the oesophagus. In successful grafts of even the earliest developmental stages the epithelia of the oesophagus, stomach and intestine have their characteristic appearance even to glands and folds. Survival is better than in the case of certain ectodermal and mesodermal rudiments (nervous tissue, kidney, heart), or of endodermal derivatives like liver and pancreas. The percentage of successful takes is high.

No typical gut differentiation appeared in grafts from embryos showing other initial organ formation (7-day), or from prerudimentary stages. However once the gut is formed, or forming, growth and differentiation continue in the foreign environment (8-day). Mass and organization thus play important parts in survival. Distortion or malformation are at a minimum in the isolated grafted material. It is concluded that the capacity of the endodermal derivatives of the mammalian embryo to grow and differentiate in adult omental grafts is very good (Nicholas and Rudnick).

175. PREOVULATORY MATURATION OF THE RABBIT'S EGG. A. J. Waterman and B. B. Owen, Williams College.

Ovaries of mated does were secured at $\frac{1}{2}$ -hour intervals following copulation to 15 hours post coitus. Two to four ovaries of each stage were sectioned entire.

Maturation is not limited to the mature preovulatory follicle (Mathis) and the time of appearance of maturation figures varies. Ovum maturity is reached before that of the follicle since small, deeply embedded follicles with very small antra may form polar bodies (Pincus and Enzmann). Only mature follicles ovulate. The ovaries of three does showed polyovular condition and these contained maturation figures. Visible evidences of maturation appear in some ova around $4\frac{1}{2}$ hours after coitus (and as late as 9 hours) in the migration of the germinal vesicle toward the periphery of the ovum. Eggs in atretic follicles may be stimulated to mature. A cytological study has been made. The polar body frequently divides mitotically to form two, with the spindle parallel to the egg surface. The structure of the polar body shows a central, coarsely granular area containing the nuclear material enclosed by a finely granular cortical portion. The outer portion of the latter is quite free of granules.

Ovulation may occur around 8 to 14 hours after coitus, average around 10 to 12 hours, as various ova in an ovary react differently to the maturation stimulus. There is a variable lag period. This accounts for the varying stages commonly found in a litter of very young embryos. No blastomere division was seen but atretic follicles often showed fragmentation of nuclei and polar bodies.

176. THE EARLY CLEAVAGE OF POLYCHOERUS CARMELENSIS. Donald P. Costello, University of North Carolina.

The cleavage of the eggs of acelous turbellaria has been studied by Pereyaslawzowa, Rapiachoff, Gardiner, Georgevitch and Bresslau, the observations of Bresslau being somewhat in disagreement with those of the four earlier workers. A study of the cleavage of the eggs of *Polychoerus carmelensis* has shown that the conclusions of Bresslau are applicable to the development of this North American representative of the group.

The cleavage is of a spiral type, which differs from the spiral cleavage of polyclads, annelids, and mollusks in that it involves duets of blastomeres rather than quartets. The formation of the polar bodies and fertilization occur before the eggs are laid. The first cleavage results in two cells of slightly unequal size. With the second cleavage each of the two cells is divided into a macromere and a micromere, the former being about four times as large as the latter. The micromeres now divide almost equally, producing a cross of four cells at the

upper pole (6-cell stage). Then each of the two macromeres divides into unequal parts, a larger basal cell and a smaller cell lying nearer to the micromere pole. This is soon followed by the unequal division of the two basal cells, to give four basal cells arranged more or less in a row (10-cell stage). By a process of emboly the two middle basal cells are forced into the cleavage cavity, beginning gastrulation. Gastrulation is completed by repeated divisions of the micromeres and the overgrowth of their products.

177. PRIMARY SEXUAL PHASES IN THE OVIPAROUS OYSTER, *OSTREA VIRGINICA*. W. R. Coe, Yale University.

Examination of more than 6000 young oysters in their first spawning period, obtained from various localities from New England to the Gulf of Mexico, has shown a high variability in the ratio of the two sexual phases in different situations and in different years. Under conditions unfavorable for rapid growth at northern localities the proportion of individuals functioning as females at their first spawning season was found to be less than eight for each 100 males, while at the same localities under more favorable nutritive conditions the female ratio exceeded twenty. Farther south the female ratio averaged about forty. The ratio may change somewhat during the first spawning season, since in the primary sexual phase female development may be either direct or indirect.

In the second and later spawning periods, the proportion of females increases with age until there are more females than males, since more of the males change to the female phase than the number of individuals which experience a sex change in the opposite direction. Change of sexual phase follows the cytolytic of most of the sexually differentiated reproductive cells after spawning, leaving mainly undifferentiated gonia as residual cells, but in many individuals cells of one or both sexual types remain. Local races with differing genetic factors as well as environmental conditions may account for the diverse sexual ratios. Two generations in 1 year may occur in southern localities.

178. COALESCENCE BETWEEN COATED AND FRESH OIL DROPS. M. J. Kopac, Washington Square College, New York University.

The tendencies of drops which have been in contact with cytoplasm to coalesce with other drops were tested. Mineral oil (A) drops with which sea urchin eggs coalesce do not form visible interfacial films. Frequently the outermost portion of the drop's surface is covered only by a very thin layer of cytoplasm. Oleic acid (B) causes immediate cytolysis of the surrounding cytoplasm and the drop becomes coated completely by a granular coagulum, frequently 7 to 10 μ thick. Shortly thereafter this drop is partly or completely ejected by the cell. Coated B drops coalesce with A provided only a short time (3 to 5 minutes) elapses after their ejection, but they do not coalesce with B. Older coated drops will not coalesce with A due to the rigidity of the granular coagulum. The coated drops always engulf the fresh drops. A drops which are either partly exposed or surrounded by a very thin layer of cytoplasm coalesce instantly with either A or B. Immediately after the in situ A drops coalesce with B, a cytolytic reaction follows together with the formation of a granular coagulum around the drop. This is a typical oleic acid reaction, and it shows that the oleic acid is sufficiently surface active to accumulate at the surface of the mineral oil. The granular coagulum surrounding the oil drop, which does not form unless cytolysis occurs, appears to be largely composed of insoluble proteins.

179. THE RATE OF PROTOPLASMIC STREAMING IN ELODEA CELLS AT HIGH HYDROSTATIC PRESSURE. Douglas Marsland, Washington Square College, New York University.

Whole leaves of *Elodea canadensis*, immersed in their natural medium within a pressure chamber which permits microscopic study, were subjected to pressures ranging from 1 to 550 atmospheres. In each experiment the rate of streaming under increased pressure, relative to the rate at atmospheric pressure was obtained by timing the progress of the chloroplasts in passing from one point to another in a selected cell or cells. With increasing pressures the rate of streaming regularly diminishes, reaching zero at 450 to 500 atmospheres. When decompressing, at each lower stage of pressure there is a return approximately to the previous rate at this stage, provided the highest degree of compression is not maintained for more than about 25 minutes. When the rate, calculated by averaging 100 measurements at each of the pressure stages, is plotted against the pressure, the curve closely resembles, in form and placement, the results previously obtained in studying the effects of pressure upon the protoplasmic viscosity of the *Amoeba* and of the *Arbacia* egg, and upon the rate of furrowing in these eggs. This suggests that the pressure is affecting similar components in these diverse protoplasmic systems. The simplest inference is that it produces a shift in the sol-gel equilibrium of the protein components.

180. THE VISCOSIMETER METHOD FOR DETERMINATION OF CELL CONCENTRATION IN SUSPENSIONS OF LIVING CELLS. Herbert Shapiro, Princeton University.

The equation derived by Einstein for the effect on the viscosity of a liquid of adding a suspension to it states that

$$\eta_s = \eta_o(2.5\phi + 1)$$

where η_s and η_o are the viscosity of suspension and of medium respectively, and ϕ is the volume of suspended material in unit volume of suspension. Preliminary experiments have been made with cell suspensions, diluted by various amounts, to determine whether the time required for flow out of a viscosimeter could be utilized as a measure of cellular concentration. For beef erythrocytes, and for *Asterias* eggs (whose jelly differs from that of *Arbacia* in being firmly adherent to the cell) it was found that there is a definite relationship, which follows the form of the equation just cited, and is quite precise. For example, the time required for emptying of a suspension of beef erythrocytes, diluted to one-sixth original concentration, was in duplicate determinations, 61 seconds, compared with 251 seconds for defibrinated whole blood. Similar figures for a suspension of *Asterias* eggs were 68.7 and 684 seconds. Thus for relative cell concentrations this procedure has a definite utilizability. It is planned to explore the limitations of this method as an absolute one by checking it against procedures previously studied in detail, and to extend the study to other material, such as bacteria.

181. ENZYME ACTIVITIES IN RELATION TO FERTILIZATION. William Doyle, Bryn Mawr College.

The catalase and peptidase activities of the eggs of the sea urchin, *Psammechinus miliaris*, were determined before and after fertilization with respect to the presence of membranes surrounding the eggs. The work was carried out at the Carlsberg Laboratory, Copenhagen by means of the Linderstrøm-Lang and Holter technique. Fertilized eggs with intact membranes showed lower peptidase activities than unfertilized eggs. Fertilized eggs with disintegrated membranes showed the same peptidase activities as unfertilized eggs. The degree of cytolysis and the completeness of formation of the fertilization membrane affects the apparent peptidase activity of the egg. The catalase activities of fertilized and unfertilized eggs are the same regardless of the presence of the membranes.

182. THE RESPIRATORY METABOLISM OF BLUE CRAB NERVES (*CALLINECTES SAPIDUS*). Verlus F. Lindeman, Syracuse University.

In three series of experiments, sections of the pincher and first walking appendage nerve were studied for oxygen consumption. The respirometer used was a modified type of the Fenn monometer.

The first two series consisted of measurements on the pincher nerves at temperatures of 20.4°C. and 25.8°C. respectively. In series I the paired nerves of ten animals averaged 102.25 cc. of oxygen per gram weight per hour, with extremes between different animals of 72.27 and 152.32 cc. oxygen per gram weight per hour, and a mean difference between pairs of the same animal of 5.32 cc. per gram weight per hour.

In series II, eleven animals tested gave an average of 136.40 cc. of oxygen per gram weight per hour, with extremes between different animals of 97.57 and 186.40 cc. oxygen per gram per hour, and a mean difference between pairs of the same animal of 6.20 cc. oxygen per gram weight per hour.

In series III, the nerves from the pincher and first walking appendage was tested for 11 animals at a temperature of 20.4°C. Nerves from the same side of the animal were used. The average oxygen consumption for the walking appendage nerve was found to be 156.29 cc. per gram per hour, while the average for the pincher nerves for the same animals was 99.10 cc. oxygen per gram weight of nerve per hour.

183. A COMPARATIVE STUDY OF THE ADDUCTOR MUSCLE OF THE CRAYFISH AND SARTORIUS MUSCLE OF THE FROG; WITH SPECIAL REFERENCE TO THE ACTION OF ELECTROLYTES IN RESPONSE TO ELECTRICAL STIMULATION. Josephine Lamb Fernandes, Syracuse University. (Introduced by V. F. Lindeman.)

An apparatus was devised whereby the contractions of the adductor muscle of the crayfish and sartorius muscle of the frog could be perfused with various iso-osmotic solutions. Crayfish muscle showed spontaneous contractions in response to isotonic NaCl and KCl. These spontaneous contractions always occurred after submaximal induced stimuli. No spontaneous contractions were noted in response to CaCl₂. The sartorius muscle of the frog displayed slight spontaneous twitches in response to the isotonic KCl only.

LiCl was successfully substituted for NaCl in the normal perfusing solution for both the crayfish adductor muscle and sartorius muscle of the frog. Therefore, it was concluded that in both the skeletal muscle of the amphibian and the adductor muscle of the crayfish, LiCl was capable of replacing NaCl in its action upon the muscle.

The application of small amounts of KCl to the adductor muscle of the crayfish produced immediate spontaneous contraction. Repeated stimulation of the muscle while it was being perfused with isotonic KCl, produced increased tone which was equivalent to contracture.

Both CaCl_2 and SrCl_2 were found to relax the muscle of the crayfish after increased tone had been induced by the action of the K ion. Similar experiments on the frog's sartorius muscle produced the same relaxation. It would appear that the effects of CaCl_2 and SrCl_2 are analogous on the invertebrate and vertebrate muscle.

In general it may be said that the adductor muscle of the crayfish is more susceptible to the effects of various electrolytic ions than is the amphibian skeletal muscle.

184. THE ACTION OF ACETYLCHOLINE AND OF INHIBITORY NERVES UPON THE HEART OF VENUS. C. Ladd Prosser and Hazel B. Prosser, Marine Biological Laboratory and Clark University.

The heart of the clam, *Venus*, is extremely sensitive to acetylcholine. Sensitivity is greater early in the summer than later: threshold concentration is 1 in 10^{12} in June, and 1 in 10^9 in September. The action is mainly a negative inotropic one, but the rate of beat is also slightly slowed. Eserine increases the sensitivity to acetylcholine.

Observation of ventricles inhibited by acetylcholine shows that conduction is not stopped—contraction in response to mechanical stimuli also continues. There is no localized pacemaker, but the beat can start at any point on the surface of the ventricle.

Stimulation of the visceral ganglion inhibits the heart much as does acetylcholine except that a much more marked fall in tonus is set up by the nervous inhibition than by acetylcholine. Reflex inhibition can be caused by stimulation of the mantle and other regions. This natural inhibition is not increased nor is its threshold lowered by the application of eserine or acetylcholine to the heart. It is possible, however, to transfer sea water from the heart and pericardium of an inhibited preparation to a normally beating heart, and to get inhibition of the beat in the second heart.

It is concluded that the normal inhibition of the clam heart is mediated by a substance which may be similar to but is not identical with acetylcholine.

185. THE ACTION OF ACETYLCHOLINE ON THE SKELETAL MUSCLE FIBERS OF THE FROG. Elsa M. Keil and F. J. M. Sichel, New Jersey College for Women and College of Medicine, University of Vermont.

We have previously shown that the application or injection of very small amounts ($5000 \mu^3$) of acetylcholine in concentrations ranging from 1 in 10^3 to 1 in 10^{11} had no effect on the isolated single fiber (adductor, *Rana pipiens*), beyond

effects attributable to the solution medium. This is confirmed for single fibers isolated from the sartorius of the frog. However, if the sartorius be dissected from the frog, and even if the pelvic and tibial ends be both cut across, acetylcholine will cause a propagated twitch when applied as a small droplet with a micropipette to the dorsal surface of the muscle in the region of the nerve twigs. This twitch is observed only in those fiber bundles in the vicinity of the point of application of the acetylcholine. If either the pelvic or tibial half of the sartorius is split lengthwise into two parts, one of which contains about twenty fibers, then acetylcholine applied to the surface of this bundle also causes a similar propagated twitch response. If the bundle is split into still smaller groups, a degree of subdivision will eventually be reached such that acetylcholine will no longer evoke twitches in fibers still irritable to electrical stimulation. This apparently occurs when the subdivision has interfered with the nerve supply.

We conclude that these experiments offer further evidence in support of our previous statement that the action of acetylcholine is not upon the contractile mechanism of the muscle directly. We further conclude that acetylcholine evokes twitches in the sartorius muscle of the frog only when the terminal nerve supply or possibly some junctional or receptor tissue is intact. These conclusions are in accord with Garrey's experiments on turtle and *Limulus* heart, and with Armstrong's experiment on embryonic *Fundulus* heart.

186. A CRUSTACEAN NEUROHUMOR. John H. Welsh, Harvard University.

The perfusion or extraction of crustacean nerves with sea water or crab-Ringer yields an active substance which has marked physiological effects on the intact or isolated crustacean heart. An extract of 0.5 mg. of nerve by wet weight, per cubic centimeter of perfusing fluid, is sufficient to more than double the rate of beat of the isolated heart of *Maia*, the European spider crab; while 2 to 5 mg. per cubic centimeter causes a general tonic contraction of the heart with an abnormally high frequency of beat and, if perfusion continues, the heart finally stops in systole. The normal beat is rapidly restored by washing. The effects of this nerve substance can be reproduced with potassium but much higher concentrations of K are necessary than those contained in the tissue extracts. It is possible that the nerve substance produces its effects by the release of bound K in the heart tissue. The substance is soluble in water but is not soluble in ethanol. It is not destroyed by boiling alone, nor by boiling with $\frac{N}{10}$ HCl or NaOH.

Extracts of crustacean muscle yield a substance which in low concentrations causes a decrease in the frequency of heart beat and in higher concentrations stops the heart in diastole.

Since acetylcholine has not been found to play the same important role in crustacean neuromuscular physiology that it does in vertebrates and certain of the other invertebrates it is suggested that this substance, or neurohumor as it might fittingly be called, may be its counterpart.

187. SPECTRAL COMPOSITION OF THE LIGHT EMITTED BY JAMAICAN FIREFLIES.
John Bonner Buck, Johns Hopkins University.

The light emitted by thirteen species of fireflies in Jamaica, B. W. I., was analyzed spectroscopically, with the following results:

1. Spectrographically, the light emitted by all the species investigated consists of a broad structureless band lying wholly within the visible spectrum.

2. None of the fireflies studied emits light below about 5050 Å or above 6550 Å.

3. Several species emit light of almost the same spectral composition, whereas others differ considerably from one another.

4. The spectra of some of the species are relatively extensive (e.g., 5050 Å to 6450 Å), those of others are more restricted (e.g., 5400 Å to 6375 Å).

5. It was demonstrated photographically that the apparently different color of the light emitted by the thoracic and abdominal light organs of the elaterid, *Pyrophorus plagiophthalmus*, is due to an actual difference in the color of the light emitted, and not to any subjective (Purkinje) effect. The spectrum of the thoracic organ extends from 5075 Å to 6400 Å, and that of the abdominal organ from 5350 Å to 6400 Å.

188. STUDIES IN POPULATION PHYSIOLOGY. VIII. THE EFFECT OF LARVAL POPULATION DENSITY ON THE POST-EMBRYONIC DEVELOPMENT OF THE FLOUR BEETLE, *TRIBOLIUM CONFUSUM* DUVAL. Thomas Park, The University of Chicago.

Using the flour beetle, *Tribolium confusum*, an analysis was made of the effect of differential larval density on 1) the mortality and duration of the larval period; 2) the mortality and duration of the pupal period, and 3) the weight of subsequent pupae and imagoes. It was shown that as density increased more larvae and pupae died and the duration of metamorphosis was extended. There was also a suggestion that larvae and pupae reared in crowded cultures weighed less than those reared under conditions of isolation. The nature of the density effect was analyzed. The conclusion was reached that, for this case, it is the effect the larvae have on their medium that is important in bringing about the effects noted and not the behavioristic interactions between individuals themselves. These facts have certain implications for the dynamics of populations.

189. A NOTE ON THE SIZE AND COMPOSITION OF OLD *TRIBOLIUM CONFUSUM* POPULATIONS. Thomas Park, The University of Chicago.

An analysis was made of the composition of 200 cultures of *Tribolium confusum* that were 1) started initially in four distinct types of flour and 2) allowed to grow unmolested for 1 year. It was shown that the total population size (i.e., after 1 year) was in inverse ratio to the initial conditioned flour (flour altered by the beetles' activity) content of the medium. The quantitative distinctions between the four experimental series were due largely to differences in the number of imago and larval beetles and not to the eggs and pupae.

190. GROWTH AND SURVIVAL OF JAPANESE BEETLE LARVAE REARED IN DIFFERENT MEDIA. Daniel Ludwig and Henry Fox, University College, New York University.

Japanese beetle larvae were reared in various media at 25°C. and their rates of growth and percentage of survival during the first two instars compared. The media used were: soil; plant molds derived from *Andropogon*, *Carex* and *Osmunda*; *Andropogon* mixed with soil; *Sphagnum* moss; and commercial peat. Each was used alone and with the addition of wheat grains (living or killed by heating to 100°C.), or a suspension of yeast.

Soil and *Carex*, each containing living wheat, gave most favorable results. In the former, 96% survived to the third instar, the duration of the first instar averaging 13.7 days, and the second 17.7 days; while in the latter, the corresponding figures were 100%, 18.1 days, and 16.8 days. No differences were noted in the duration of the first instar when reared in *Andropogon*, *Andropogon* and soil, *Osmunda*, *Carex* or peat, when these media were used with living wheat; while the second instar was significantly shorter in *Carex* and in soil. *Sphagnum*, alone or with wheat, was unsatisfactory, the larvae surviving 7.6 days; while in peat, they lived 10.3 days and in soil 26.6 days. The addition of wheat to a medium improved it, living wheat being definitely more favorable than dead wheat. Thus, peat with living wheat gave a survival of 52.0% to the third instar, and with killed wheat of only 34.6%. The addition of yeast also made the medium more favorable, 42.6% surviving to the second instar in soil and yeast, and 8% in peat and yeast.

191. THE CAUSE OF THE RED-GREEN COLOR CHANGE IN *EUGLENA RUBRA*. Leland P. Johnson and Theodore L. Jahn, Drake University, State University of Iowa and the Iowa Lakeside Laboratory.

Euglena rubra is one of the species of *Euglena* in which a color change from green to red may be produced in a few minutes by the migration of pigment granules from central to peripheral positions. The purpose of the present study was to determine the color of light most effective for bringing about this change.

Light sources used were direct sunlight and a 500 watt lamp. The organisms were placed between a glass slide and cover slip, and the color change was observed macroscopically and microscopically.

When the experimental arrangement permitted heating of the slide to occur, the time necessary for the change was an inverse function of energy content as measured with a thermopile, regardless of the color of the light employed. This color change could also be produced by heating to about 40°C. in the dark. However, when heating was minimized by placing the slides on ice, blue light was much more effective than longer wave lengths of the same energy content. At low temperatures the end point of the color change was also much sharper than when heating of the slide was permitted. The green-red color change, therefore, may be produced by either of two factors: 1) an increase in temperature produced by heat or radiant energy, or 2) by visible light, especially of the shorter wave lengths, in the absence of appreciable heating.

192. A STUDY OF THE CAPACITY-INTENSITY FACTORS IN CYANIDE INHIBITION. Wilbur Robbie, State University of Iowa. (Introduced by J. H. Bodine.)

An investigation is being made of the relative influence of the capacity (exposure time) and intensity (concentration) factors in the cyanide inhibition of the eggs of the grasshopper *Melanoplus differentialis*, using as a measure of effect the length of time (T) necessary for recovery to the original level of respiration, and the oxygen consumed during this process (O).

Preliminary investigation shows that for developing eggs: 1) When the product of the capacity (C) and intensity (I) is maintained at a constant level (K), (C) is more potent than (I): long exposures at low concentrations are more injurious than short exposures at high concentrations. 2) As (K) increases (T) and (O) increase in a roughly proportional manner, depending upon the relative values of (C) and (I). 3) (O) appears to vary directly with (T): the longer the time necessary for recovery the more oxygen is consumed during the process.

Further investigation is being made to determine: 1) the relationship of the (C) and (I) factors during the blocked or diapause condition; 2) the correlation between oxygen consumption and morphological development after exposure to cyanide.

193. THE RESPONSES OF TWO SPECIES OF OPOSSUMS TO SELECTED STIMULI. Elbert C. Cole, Williams College.

1. Comparative studies were made of the behavior of two Central American species of opossums: *Metachirus opossum fuscogriseus* (Allen), and the brown opossum, *Metachirus nudicaudata dentaneus* (Goldman).

2. The reactions of Allen's opossum were violent and aggressive. Respiration was rapid and a loud and continuous hissing was characteristic of this animal so long as the observer was near. Food was promptly snatched and held. In the presence of chloroform fumes this animal seemed unable to modify its respiratory behavior and usually succumbed in about $2\frac{1}{2}$ minutes.

3. Although about the same size as Allen's opossum, the brown opossum appeared to be far less aggressive. It opened its mouth widely, but its hissing was gentle and it made no violent charging movements. Food was ignored. Chloroform fumes produced anesthesia in about $4\frac{1}{2}$ minutes, but a more or less continuous exposure was required to maintain this condition.

4. Both sexes reacted to these stimuli in similar ways.

5. Local information indicated that the apparent docility of the brown opossum was deceptive; that in encounters between the two species the brown opossum was usually the victor.

194. CONCERNING THE EFFECT ON HONEYBEES OF LEAD ARSENATE, CALCIUM ARSENATE, AND PHENOTHIAZINE AS STOMACH POISONS. Lloyd M. Bertholf, Western Maryland College and United States Department of Agriculture.

Honeybees were fed individually and in a single dose various amounts of the three insecticides mentioned and were then confined in lots of 100 in cages in incubators maintained at 29°C. and 40% relative humidity where they had continuous free access to both sugar syrup and water. It was found that calcium

arsenate was most toxic, reducing the longevity slightly when doses containing only 0.05, 0.1, 0.2 and 0.4 micrograms of elemental arsenic were given, but showing a very decided effect when 0.8 micrograms or more were fed. Lead arsenate showed practically no effect when 0.05 micrograms were fed, increased slightly for doses containing 0.1, 0.2, 0.4, 0.8 and 1.6 micrograms, then increased decidedly for heavier doses. Whereas a dose of calcium arsenate containing 1.6 micrograms of elemental arsenic was sufficient to kill the average bee in less than 24 hours, it took doses containing 51.2 micrograms of arsenic in the form of lead arsenate to give the same lethal effect. Phenothiazine, however, had very little toxic action at all. Bees fed doses containing several times as much as they would ordinarily get from a commercial spray mixture lived practically as long as the controls, i.e., about 20 days. Further experiments have indicated that both lead and calcium arsenate are slightly repellent to bees, since they take up plain sugar syrup somewhat more readily than the same syrup containing either of these insecticides.

195. SPECIFICITY OF THE PITUITARY GONADOTROPIC FACTORS WITHIN THE AMPHIBIA.

Charles W. Creaser and Margaret Sheolnek, Wayne University.

Ovulation out of season has been brought about in *R. pipiens* only by homoplastic implants or by implants of glands from certain other members of the genus *Rana*. Tests made with a large number of glands from the phylogenetically more primitive amphibian, *Necturus*, fail to induce ovulation in frogs in which ovulation was known to be possible using the same technic and frog pituitaries. This fact sets the limits of specificity within the amphibian group. It would seem that either the *Necturus* glands are of very reduced potency or that a very sharp specificity is shown by *R. pipiens* for this type of preparation.

196. A COMPARATIVE STUDY OF THE NORMAL RELATIONSHIP BETWEEN PITUITARY AND TESTIS IN THREE VARIETIES OF FOWL. W. R. Breneman, Indiana University.

The normal body, testis, and comb growth is studied in three varieties of chicks during the period from hatching until the thirtieth day. The testes and combs of White Leghorn chicks grow more rapidly than those of the Rhode Island Reds and the Brown Leghorn chicks present an intermediate picture. There is considerable individual variation in the testis and comb growth of White Leghorns amounting to about 300%, but this variation is less marked in the other varieties. The data indicates that comb growth is influenced by secretion of the testis at a very early date in the White Leghorn (heterogonic growth) but in the other two varieties, especially the Rhode Island Red, comb growth is proportional to body growth (isogonic growth). The evidence indicates that there is an earlier physiological activity of the pituitary in the White Leghorn chick.

197. THE EFFECT OF ESTROGENS ON THE FISH GONAD. Philip Berkowitz, Washington Square College, New York University. (Introduced by Robert Gaunt.)

In a previous report we have shown that of sixty-five fish (*Lebistes reticulatus*) treated with estrogens (theelin and theelol) from birth for periods of 1 to 5 months, all failed to show male secondary sex characters during the period of treatment although they had reached the age and size at which sexual differentiation should have been completed. The size and shape of these fish approached that of the female sex; the coloring and condition of the anal fin were entirely those of the female.

More recently the gonads of these and a second group of fish have been histologically examined. The male gonad shows two tendencies as a result of hormone treatment. In one group there is apparent not only an inhibition of spermatogenesis but a definite tendency toward involution of the gonad. In a second group, a majority, there is apparent a development of an ovotestis in which male and female gametic areas are indiscriminately scattered throughout the gonad. The latter are characterized by the presence of large rounded cells containing a vesicular nucleus whose chromatin shows nucleolar condensations. The size of these egg cells varies greatly, the largest, however, being approximately one-half the size of those of the 3-month-old control females.

An examination of the gonads of animals treated after maturity, presents in some instances features which, with a few exceptions, are similar to the above, there being gonads containing definite ovarian tissue close to areas of clearly defined spermatogenic tissue.

198. A QUANTITATIVE STUDY OF EXPERIMENTALLY INDUCED HEAT IN THE SPAYED GUINEA PIG. Vincent J. Collins, John L. Boling and William C. Young, Brown University and Yale University.

Spayed guinea pigs were injected with 50 I.U. oil-soluble theelin (Parke, Davis) followed at varying intervals from 12 to 108 hours by single injections of 0.2 I.U. progesterone (Proluton-Schering). Heat was determined by the copulatory response. The percentage of animals coming into heat in each group was plotted against the interval between theelin and progesterone injections. Length of the interval, animals injected in each group, and percentage brought into heat follow: 12 hours, 13 injected, 7.7% in heat; 18 hours, 15 injected, 20% in heat; 24 hours, 39 injected, 79.5% in heat; 30 hours, 28 injected, 78.5% in heat; 36 hours, 30 injected, 53.3% in heat; 42 hours, 30 injected, 50% in heat; 48 hours, 43 injected, 34.9% in heat; 54 hours, 43 injected, 34.9% in heat; 60 hours, 32 injected, 37.5% in heat; 72 hours, 32 injected, 34.3% in heat; 84 hours, 34 injected, 9.1% in heat; 96 hours, 28 injected, 6.9% in heat; 108 hours, 30 injected, 6.6% in heat.

Records of elapsed time between progesterone injection and first heat response (latent period) show a close inverse correlation with length of heat. When the latent period was long, heat was short and vice versa. Length of latent period and heat together approached a constant of 11.83 hours; range 10.61 hours to 13.16 hours even when few animals came into heat. This suggests that as long as theelin conditioning is sustained it is sustained in its entirety. Also, a possible assay method for oestrogens is indicated.

199. THE VAGINAL SMEAR PICTURE ACCOMPANYING THE OESTRIN-PROGESTERONE PRODUCTION OF HEAT IN THE SPAYED GUINEA PIG. John L. Boling, Vincent J. Collins and William C. Young, Brown University and Yale University.

As noted in the abstract by Collins, Boling and Young, some animals injected with 50 I.U. of oil-soluble theelin (Parke, Davis & Company) become conditioned for heat within 12 hours after injection and some remain conditioned until after the 108th hour.

Vaginal smears made at the beginning and end of 132 of these experimentally produced heat periods have been examined. Prior to the forty-eighth hour the smears are predominantly dioestrous. Subsequent to the fifty-fourth hour they tend to resemble those made during the heat periods of normal animals although it is not until after the seventy-second hour that fairly typical cornified smears are found.

Since heat was produced most frequently by progesterone (Proluton-Schering) injected 24 or 30 hours after theelin administration when the vaginal smear is still dioestrous and the vaginal smear picture approaches those ordinarily found during heat only after the fifty-fourth hour, it is clear that conditioning for heat is accomplished more quickly than vaginal cornification. This conclusion is consistent with the circumstance that normal females are conditioned for heat as early as the twelfth and thirteenth day although vaginal changes occurring about the time of heat appear 3 or 4 days later. In addition, further evidence is given that vaginal changes and heat are separable.

200. THE RELATION OF DECIDUOMATA TO THE REPRODUCTIVE CYCLE IN THE GUINEA PIG. Edward W. Dempsey, Brown University and Harvard Medical School. (Introduced by A. D. Mead.)

It is well known that during pregnancy suspension of ovulation and persistence of the corpus luteum occur. The corpus luteum maintains the uterus in its pregnant condition and likewise suppresses ovulation, but the factors which account for the maintenance of the corpus luteum of pregnancy are not well understood. It is generally believed that there is a mutual interaction in which the fetus and placenta are dependent upon the corpus luteum which in turn is maintained by some substance or substances produced in the fetus, fetal placenta or maternal placenta.

That this substance is not produced by the maternal placenta is indicated by the following experiment. Deciduomata were induced by threading the uteri of nine female guinea pigs on the sixth day after heat. Eight of the nine came into heat 14 to 19 (average 15.2) days after the last period, despite the presence of decidual tissue which was determined at autopsy 24 hours after heat. The regressing corpora lutea of the preceding cycle and the presence of recent ovulation points in each of these cases show that the decidual tissue did not suppress ovulation or cause the persistence of the corpus luteum. It is concluded that the substance responsible for these phenomena during pregnancy is not produced by the maternal placenta, and it is suggested that the fetal tissue may be the site of its origin.

201. EFFECTS OF TESTIS REMOVAL ON THE FIBRO-MUSCULAR SCROTAL SAC IN THE ALBINO RAT. L. J. Wells and M. D. Overholser, University of Missouri.

This paper reports the results of some preliminary experiments. Testes of twenty-nine adult rats were unilaterally or bilaterally removed, and in some cases a paraffin pellet was placed in the emptied scrotal sac. Following periods of 32 to 51 days, experimental rats and thirteen unoperated littermates were killed on the 130th to 321st day of life. Fibro-muscular sacs were freed of superficial fascia, severed at the inguinal ring and weighed individually preceding fixation.

In each of twenty bilateral castrates, weight of the empty sacs was approximately half as great as that for unoperated littermates. Weight of the empty sac in two unilateral castrates was 61% and 83% as great as that for the normal contralateral sac, and greater than that of the empty sac in any of the bilaterally castrated group; seminal vesicles of these two animals weighed as much as those from unoperated littermates. These data suggest that the fibro-muscular scrotal sac of the rat depends upon both hormonal and mechanical factors for its maintenance.

Weight of the sac bearing a paraffin pellet was greater than that of the empty one in each of five bilateral castrates, and even greater than that of the normal contralateral one in each of two unilateral castrates. It is unknown whether this response was due to mechanical maintenance of the sac or a specific reaction of sac tissues to a foreign body (paraffin); this question will receive further attention when microscopical preparations are completed and studied.

202. CYCLIC CHANGES IN THE THYROID AND ADRENAL CORTEX OF THE MALE STARLING, *STURNUS VULGARIS*, AND THEIR RELATION TO THE SEXUAL CYCLE. J. Wendell Burger, Trinity College. (Introduced by T. H. Bissonnette.)

Changes in the histology of the thyroid and the adrenal cortex which occurred between November 20th and June 15th were described in relation to the spermatogenetic cycle. The periods under study included: a) a period of testicular quiescence (November to early January), b) the spermatogenetic cycle (January to the end of May), c) testicular involution (June). The phases of thyroid activity found were: a) colloid storage (November and December), b) colloid absorption (January to early February), c) colloid secretion (February and March), d) colloid absorption (April and May), e) colloid secretion (June). Variations in the velocity and duration of these phases were noted. Starlings brought into full spermatogenesis by increased artificial illumination showed a uniformly present hyperplasia of the follicular epithelium. This latter finding is open to several interpretations. It is concluded that normal spermatogenesis is accompanied by no histological change in the thyroid that can be correlated with the whole cycle. One period of colloid resorption, however, roughly corresponded with the mating period.

Changes in the adrenal cortex can be correlated with the spermatogenetic cycle. During the period of spermatogenesis the cortex presented a uniform appearance characterized by fully elaborated spongiocytes. During sexual quiescence and sexual involution other cell types also occurred.

203. SEXUAL CONDITIONS IN *TRITURUS VIRIDESCENS*. ARTIFICIAL HERMAPHRODITISM. (FEMALE HOSTS WITH TESTICULAR GRAFTS.) A. Elizabeth Adams, Mount Holyoke College.

Adult female *Triturus viridescens* were engrafted with testes from adult males in April, July, October-November-December. Fifty-five received two testes in addition to their two ovaries, and sixty-five one testis after one ovary had been removed. Ninety-five of the 120 animals survived from 10 to 449 days. In seventy-one of these, the testes were attached to the host; in twenty-three, there was no connection and in one, union was uncertain. The highest percentage of attachment occurred in testes grafted in April (100%) and in July (88%). The attachment of fall grafts was 57%.

The testes recovered varied considerably in appearance. Their condition seemed to be dependent on several factors, such as: 1) success of attachment, 2) condition of testis at grafting, 3) condition of host at time of operation, 4) supply of pituitary hormone available, 5) period of survival and, possibly, 6) a sex factor.

Cloacas and wolffian ducts were examined for effects of male hormone from the testicular grafts. In no case was there any significant hypertrophy in the rudimentary glands of the cloaca. In a few instances there was a slight enlargement of the wolffian ducts but mechanical dilatation by fluid makes this enlargement of questionable significance. It is uncertain whether the structure of the testis was qualitatively inadequate for the production of the male hormone or whether the hormone was being produced but in too small quantities for effective stimulation of the cloacal glands and wolffian ducts.

204. TESTS OF THE POTENCY OF THYROID AND PITUITARY GLANDS OF TADPOLES OF *RANA CLAMITANS*. A. Elizabeth Adams and Olive Bartholomew, Mount Holyoke College.

The ability of the thyroid and pituitary glands of tadpoles of *Rana clamitans* with hind limbs 1 to 6 mm. long to induce molting in adult *Triturus viridescens* thyroidectomized or hypophysectomized for 4 weeks or more was tested. Implantation of one pair of thyroids from donors with hind limbs 1, 2 or 3 mm. long caused the shedding of the cornified epidermis that accumulates after thyroidectomizing newts. This reaction was usually initiated in 4 days. After the molt, the layers of cornified epidermis again piled up. Examination of the grafts at 6 to 18 days after implantation revealed that the follicles with their contained colloid gradually disappeared until almost no trace of the graft remained. However, it must have released sufficient thyroid hormone to cause the initial reaction.

Transplantation of one pituitary from a tadpole with hind limbs 2, 3, 4, 5 or 6 mm. long or of two or three glands from tadpoles with hind limbs 2 mm. long did not cause the molting of hypophysectomized newts. Histological examination of the thyroid glands of these hosts showed no stimulation of the typically inactive gland of the hypophysectomized newt.

These results indicate a marked difference in the potency of the thyroid and pituitary glands of *Rana clamitans* tadpoles with hind limbs 1 to 6 mm. long, when judged by their capacity to cause the molting reaction in thyroidectomized or hypophysectomized newts. Furthermore, grafts thyroids of anuran tadpoles seem unable to establish themselves in adult urodele hosts.

205. HORMONES AND SEX DETERMINATION IN FISHES AND IN FROGS. E. Witschi and E. N. Crown, State University of Iowa.

The addition of testosterone propionate (Ciba) to the water in which pregnant females of *Xiphophorus helleri* are kept, causes abortion or resorption within 1 or 2 days. All large eggs also undergo resorption in non-pregnant adult females. All females consequently assume ovaries that resemble testes in appearance but spermatogenesis has not been observed so far. The treated females assume gradually but completely the male secondary sex characters (coloration, copulatory anal fin, sword of the tail fin).

Two groups of twelve newly hatched larvae of *Rana pipiens* were raised in solutions of synthetic testosterone propionate (Ciba) and dihydrotheelin (Doisy) respectively. Four months later only six survived of the testosterone group, all males, and only four survived of the dihydrotheelin group, of which three were normal females and one a hermaphrodite. These experiments are being repeated on a larger scale. At the present they are of interest mainly because they fail to show the 'paradoxical' masculinizing effect of estrogens which was reported by Padoa ('36).

206. STUDIES ON THE ENDOCRINES OF TELEOSTS. I. THE MORPHOLOGY OF THE HYPOPHYSIS OF THE GOLDFISH (*CARASSIUS AURATUS*). W. Randal Bell, Washington Square College, New York University. (Introduced by Harry A. Charipper.)

The anatomical relationships and position of the pituitary in *Carassius auratus* are distinctive not only for Teleostei but for the vertebrate group as a whole. It is located well below the floor of the brain in the region of the diencephalon, above the prominent parasphenoid between the prootic and alisphenoid bones. Dorsally, it is separated from the floor of the brain by a tough membrane perforated by a cylindrical stalk which attaches the pituitary to the brain. The hypophysis is poorly vascularized as compared to that structure in the higher vertebrates. Only a few blood vessels were observed entering the gland from the vascular network in its connective tissue sac.

The gland is made up of four separate pars: a) pars nervosa, consisting of two connected, irregular masses with root-like processes ramifying throughout the rest of the pituitary; b) pars intermedia, a large compact ventral lobe separated, dorsally, from other gland tissue by a narrow cleft; c) Übergangsteil, large, thick and plate-like, dorsal to the intermedia and resembling the pars anterior of higher forms; d) pars anterior, small and plate-like, antero-dorsal in position, with a structure similar to the tuberalis of higher forms. Confirmation of Bock ('28) is offered that the Übergangsteil in Teleostei histologically, at least, is to be compared to the pars anterior of higher forms. Similarly on the basis of cell type and arrangement, position and relationship, the pars anterior of Teleostei may be compared to the pars tuberalis.

207. THE EFFICACY OF EXTRACTS FROM THE BODIES OF FUNCTIONING SUPPLEMENTARY REPRODUCTIVES OF TERMITES IN INHIBITING OR RETARDING NEOTEINIC SEXUAL DEVELOPMENT IN ISOLATED NYMPHS. S. F. Light, Olga Hartman and O. H. Emerson, University of California.

One hundred and forty groups, of ten nymphs each, of *Zootermopsis angusticollis*, were isolated, one series of twenty serving as a control, the other six series being fed each week extracts from bodies of thirty-three functioning female supplementary reproductives. Extracts were made by separating 1) viscera, 2) head with prothorax and 3) body wall, grinding each, extracting first in water and then in methyl alcohol, filtering, and concentrating by boiling. The concentrated extracts were fed on filter paper. Effects of extracts in inhibiting sexual development as indicated by pigmentation and egg laying were noted weekly.

All extracts exerted some inhibiting effect upon pigmentation. All save the water extract from viscera and the alcohol extract from body wall inhibited egg laying to a greater or less degree. Two extracts were strongly inhibitory both for egg laying and pigmentation. These were the water extract from head prothorax and the alcohol extract from the viscera. That the water extract from viscera was hardly at all inhibitory while the alcohol extract from viscera was very strongly so, and other similar discrepancies, seem to indicate that at least two different inhibitory substances are involved. Numerous discrepancies as to the relative inhibitory effect of certain of these extracts with regard to pigmentation and egg laying point to the probability that while correlated these processes are separately controlled.

208. EXPERIMENTAL PRODUCTION OF EXOPHTHALMOS IN FUNDULUS. A. A. Abramowitz and H. L. Fevold, Harvard University.

Bilateral, equal, and complete exophthalmos was produced in normal adult Fundulus by the injection of various mammalian anterior pituitary preparations. Exophthalmos occurred 5 to 7 hours after an intraperitoneal injection, became progressively more marked during the next 2 or 3 days, and subsided within 14 days, the eyeballs reverting to their normal position. The reaction, with proper dosage, occurs in 100% of the cases; the exophthalmos is always bilateral, equal and complete. The responses were produced by sheep pituitary whole gland (aqueous pyridine extract) in doses varying from 50 mg. to 1 gm. equivalents. Commercial preparations (Antuitrin S, G) were ineffective. F.S.H. but not a L.H. fraction was active. A benzoic acid soluble fraction of whole gland extract was active in 20% of the animals injected with various dosages; a benzoic acid insoluble fraction was inactive. That the response is due to the anterior lobe is indicated by the ineffectiveness of pituitrin, oxytocin, vasopressin and intermedin. Antiduretin alone was not tried.

Mammalian thyroid powder, and thyroxine were ineffective. Methyl cyanide in various concentrations, was inactive, as well as adrenalin. However, adrenalin plus thyroxine produced exophthalmos occasionally, the eyes protruding only to about half the full length.

Histological examination of exophthalmic fish showed thyroid activation. The reaction can be produced in hypophysectomized Fundulus; thyroidectomy was not attempted. The mechanism by which exophthalmos is produced in Fundulus is uncertain, but it is possible that the thyrotropic and other hormones may be involved.

209. ON THE SPECIFICITY AND RELATED PROPERTIES OF THE CRUSTACEAN CHROMATOPHOROTROPIC HORMONE. A. A. Abramowitz and R. K. Abramowitz, Marine Biological Laboratory, Woods Hole.

A minimal unit of activity of the eyestalk hormone is defined as that amount in 0.05 cc. (injection volume) which produces melanophore stellation in ten of twenty injected, eyestalk-amputated *Uca pugnator*. The calculated amount of hormone in one eyestalk is 0.2 γ , and 0.00015 γ (0.001% sea water solution of eyestalks) represents a minimal unit.

Sea water when injected into blinded crabs is without effect. Distilled and tap water are strongly active. Sucrose isosmotic with crab blood, 200% and 50% sea water are inactive. Thirty-three per cent and 25% sea water are partly active; greater dilutions are fully effective. The course of the response produced by hypotonic solutions is different from that by the hormone in any concentration. Various salts isotonic with sea water were inactive, as were sixteen different drugs in doses of 100 γ . Blinded animals placed in distilled water darken, but become refractory several days later. Isolated leg melanophores show irregular responses to various ions, but contract to sodium or potassium. Sea water extracts of various internal organs (0.1% solution) of normal and blinded animals were inactive. Extracts of entire bodies (excepting eyestalks) of normal and blinded 3 weeks previously were active in a 2% solution.

The hormone is stable and somewhat potentiated by dilute acid at 100°C. for 30 minutes. Activity is totally destroyed by dilute alkali in 10 minutes at 100°C., and cannot be regenerated by acid. The hormone can be concentrated ten times by repeated 95% methanol extraction of a 66% pyridine soluble fraction of a distilled water extract.

210. ON THE NATURE AND THE EFFECTS OF A HORMONE-LIKE SUBSTANCE PRODUCED ON THE SPERMATOOA OF THE OYSTER. T. C. Nelson and J. B. Allison, Rutgers University.

Effects similar to those of certain hormones are produced by oyster sperm. Galtsoff ('30) showed stimulation of spawning in the oyster, and Nelson ('35, '36) demonstrated marked increase in water flow in the oyster by addition of oyster sperm. The hormone of Galtsoff diffuses through a collodion sac and is non-specific. Our substance will not dialyse through a collodion bag and is apparently specific, there being no effect on rate of water flow in the oyster with sperm of *Venus mercenaria*, *Modiolus demissus*, *Nereis limbata*, with human sperm, nor with glutathione. Degree of response depends upon the amount of substance introduced, does not follow all or none principle. The active substance is attached to the spermatozoa; it is not dissolved or suspended in the fluid expressed with the sperm from the testis. This substance is stable in the presence of sodium chloride and ammonium sulfate, but it is easily destroyed by heat, acids, alkalis, acetone, and other protein denaturants. The activity preserved for 4 months by quick freezing and maintaining in the frozen state is destroyed if thawed and frozen again. The properties of the active principle are similar to those of a labile protein. It is possible to obtain from the sperm an insoluble protein preparation which carries the reactive groups producing the characteristic effects on water flow in the oyster. The data suggest therefore that the spermatozoa secrete a compound protein membrane carrying specific labile groups which initiate the response.

211. NORMAL AND LASTING SEXUAL ACTIVITY DUE TO BREPHOPLASTIC GRAFTS OF THE HYPOPHYSIS IN HYPOPHYSECTOMIZED FEMALE RATS. Raoul M. May, University of Paris.

Brephoplastic grafts of the hypophysis of newborn rats into the anterior chamber of an eye of young female rats hypophysectomized a month previously causes, within a few days, growth, reappearance of the normal aspect of the coat of hair

and of the activity of the host. Oestrus appears 8 to 11 weeks after the grafting operation, and sexual cycles then follow at regular and normal intervals. Grafted females have normal ovaries, are fecund, and may give birth to young. After extirpation of the eye which bears the graft, then constituted by adult hypophyseal tissues, there follows a very limited number of sexual cycles, after which the female rat relapses into constant dioestrus; her ovaries atrophy, the corpora lutea disappear, and the follicles become atretic.

A hypophysis grafted while still in the embryonic state thus has a complete vicarious action on hypophysectomized female rats.

212. VARIATIONS IN CHICK TESTES. Frederick N. Andrews and Virgene Warbritton, Agriculture Experiment Station, University of Missouri and United States Department of Agriculture, cooperating.

The variability of chick testes (White Leghorns purchased from a hatchery handling eggs from R.O.P. flocks) was so great that it was necessary to abandon the project for using them as assay animals for gonadotropic hormones. The chick weights at 7 days ranged from 35 to 62 gm.; the testes weights of uninjected chicks, from 4 to 20 mg.; and the testes weights of injected (gonadotropic substances) chicks, from 5 to 33 mg. The chick weights at 10 days ranged from 37 to 78 gm.; the testes weights of uninjected chicks, from 9 to 23 mg.; and the testes weights of injected chicks, from 6 to 37 mg. Large testes appeared in all chicks injected with approximately 25 R.U. of gonadotropic hormone, although the heaviest testes from an uninjected chick exceeded the weights of smallest testes from a chick injected with 25 R.U. Some chicks seemed to respond to 10 R.U. but none to 5 or 1 R.U. No histological differences between testes of similar weight from injected and uninjected chicks nor between testes of even quite different weights appeared. The two testes of a chick might differ greatly in size and weight and but little in histological appearance. The chicks could be used for making pregnancy tests on mares but not for bioassay of small quantities of hormone in the serum of non-pregnant mares. The groups were not segregated, and it is possible that the uninjected group could have obtained some hormone from the droppings of the injected chicks.

213. THE EFFECT OF TETANIZING SHOCKS ON *PARAMECIUM* AND *UROLEPTUS*, WITH REFERENCE TO INJURY AND RECOVERY. I. A. Tittler, Brooklyn College. (Introduced by B. R. Coonfield.)

The organisms to be exposed were placed in a small glass-bottomed 'tetanizing' chamber lined with paraffin, to which electrodes from an inductorium were attached. The entire chamber was placed on the stage of a binocular microscope and observed during and following the period of stimulation. The minimal lethal doses for *Paramecium caudatum* and *Uroleptus mobilis* were determined. Individual organisms were removed and isolated during exposure for future observation. Others were fixed and stained at regular intervals. Under the conditions of these investigations a 90-second exposure proved lethal for *Uroleptus*. Much less exposure was sufficient to kill *Paramecium*. Under the influence of the current, a reduction in size and change in shape occurred in both organisms.

Abnormalities in the movements of the organisms were noted. Such abnormalities were particularly evident in *Uroleptus*. Marked vacuolization of the cytoplasm occurred followed by disruption and disintegration of the cell bodies. Cases of recovery in *Paramecium* removed at the onset of vacuolization and disintegration were very few, and in no instances did recovery occur when the nuclear apparatus was affected. *Uroleptus* gave more striking results. In this organism many of the macronuclei underwent granular disintegration accompanied by disfiguration and absorption of some of the micronuclei. Recovery and regeneration of missing parts in fragments of *Uroleptus* disrupted by the current, were accompanied by profound reorganization changes comparable to those occurring at the time of division.

214. (Paper withdrawn.)

215. THE RATE OF PULSATION AND THE FUNCTION OF THE CONTRACTILE VACUOLE IN *PARAMECIUM MULTIMICRONUCLEATUM*. III. RELATION BETWEEN FEEDING AND THE RELATIVE RATE OF PULSATION OF THE ANTERIOR AND POSTERIOR CONTRACTILE VACUOLES. John A. Frisch, Johns Hopkins University and Canisius College.

In flourishing cultures of *Paramecium multimicronucleatum* the rate of pulsation of the posterior contractile vacuole was higher than that of the anterior vacuole in approximately 90% of the individuals observed. This percentage was decreased to 70 in depleted cultures, to 60 in toxic cultures, to 57 and 45 in individuals in the 3- and 4-vacuole stage of fission respectively, and to 0 in non-feeding individuals. The results indicate that the relative rate of pulsation of the anterior and posterior contractile vacuole depends upon the extent and the rate of feeding.

216. *PHACUS QUINQUEMARGINATA*, SP. NOV. Theodore L. Jahn and Fae M. Shawhan, State University of Iowa, Drake University and the Iowa Lakeside Laboratory.

The name *Phacus quinquemarginata* sp. nov. is proposed for a new species of *Phacus* with the following characteristics:

Cell flattened with concave sides and raised edges and one large keel so that in end view five longitudinal ridges separated by deep furrows are seen, three ridges on one side of the gullet and two on the other. Body spirally twisted in longitudinal axis for 90° or more; tail short as in *Ph. pleuronectes*. Flagellum $1\frac{1}{4}$ to $1\frac{1}{2}$ times body length. Stigma prominent and on the side of gullet with three ridges. Paramylum bodies discoid; two in number. Length, 38 to 40 μ ; width 32 to 34 μ .

Distribution: canals connected with Lake West Okoboji, northwestern Iowa.

217. THE QUESTION OF AUTOTROPHIC NUTRITION IN THE PLANT-LIKE FLAGELLATES, CHLOROGONIUM AND EUGLENA. R. P. Hall and H. W. Schoenborn, New York University.

Attempts were made to grow *Chlorogonium euchlorum*, *Euglena anabaena* and *Euglena deses* in several inorganic media. *Chlorogonium* has been carried through six to seventeen successive transfers in different media, and in the oldest strain—now in the seventeenth transfer—the peptone carried over from the original stock culture has been reduced through serial dilution to less than 3×10^{-26} gm. per cubic centimeter. Obviously, *Chlorogonium euchlorum* is facultatively autotrophic, as reported by Loefer ('34). On the other hand, neither species of *Euglena* has grown beyond the second or third transfer in any of the solutions tested. Hence, we have failed to obtain any evidence that *Euglena anabaena* and *Euglena deses* are autotrophic flagellates.

218. EFFECTS OF DIFFERENT CONCENTRATIONS OF SODIUM ACETATE ON GROWTH OF EUGLENA STELLATA. R. P. Hall, New York University.

In attempts to improve culture media for maintenance of bacteria-free strains of *Euglenidae*, concentrations of sodium acetate favorable to growth of *Euglena stellata* were determined. Concentrations ranging from 0.003 to 0.82% were tested on this species in light and in darkness, acetate-free media being used as controls. In both light and darkness growth was most vigorous in 0.05% sodium acetate. In cultures exposed to light, growth was less than in the controls in acetate concentrations above 0.2%. In darkness, unfavorable effects of the higher concentrations of acetate were much less noticeable. This unfavorable effect of certain concentrations of acetate offers a possible explanation for the difficulties of certain investigators who have attempted unsuccessfully to grow *Euglenidae* in inorganic solutions plus sodium acetate.

219. VITAMIN B₁ AND GROWTH OF PROTOZOA. Alfred M. Elliott, Minnesota State Teachers College.

Natural crystalline vitamin B₁ Merck was shown to accelerate growth of *Colpidium striatum* when cultivated in artificial media. Basic media containing 1.0% Difco tryptone supported good growth and when supplemented with vitamin B₁ in concentrations of 1:25,000 the volume of packed organisms per 10 cc. of medium was 42.0 cu.mm. as against 33.0 cu.mm. in the control. Sterilizing the basic medium in the autoclave 45 minutes at 20 pounds pressure at pH 9.6 did not destroy all the growth promoting substance which is naturally found in tryptone. In such a medium growth occurred to the extent of 1.5 cu.mm. of packed cells per 10 cc. of medium during the same period of incubation. Autoclaved tryptone media supplemented with vitamin B₁ demonstrated an acceleration in growth of 4.5 times that in the control. Growth differences were not as pronounced when yeast extract was employed as a protein base although the same relationship was indicated. Parallel experiments with *Euglena gracilis* demonstrated a complete absence of any effect with vitamin B₁.

220. PLANT HORMONES AND GROWTH OF *EUGLENA* IN RELATION TO LIGHT. Alfred M. Elliott, Minnesota State Teachers College.

Euglena gracilis was cultured in both light and darkness in media containing the following plant hormones in concentrations of 1:10,000,000: 3-indoleacetic acid; gamma-3-indolebutyric acid; and alpha-naphthaleneacetic acid. After 10 days of incubation in a constant source of artificial illumination the following growth records were made: basic control medium (0.5% Difco tryptone solution made up with artificial tap water) = 35.0 cu.mm. of packed organisms per 10 cc. of medium; control supplemented with indoleacetic acid = 40.0 cu.mm.; control supplemented with indolebutyric acid = 47.5 cu.mm.; control supplemented with naphthaleneacetic acid = 50.0 cu.mm. Those grown the same length of time but in darkness showed the following counts: basic control medium = 326,000 organisms per cubic centimeter of culture medium; control supplemented with indoleacetic acid = 296,700; control supplemented with indolebutyric acid = 322,600; control supplemented with naphthaleneacetic acid = 280,700. Acceleration of growth of *Euglena* with these plant hormones occurred in light but in darkness no appreciable change was observed.

221. THE INCIDENCE OF PROTOZOAN PARASITES OF FROGS OF THE OKOBOJI REGION. William R. Bradley, State University of Iowa and Iowa Lakeside Laboratory. (Introduced by H. W. Beams.)

Four hundred and twelve adult frogs and 100 tadpoles were examined for parasitic protozoa. Protozoa found were: *Opalina ranarum*, *Nyetotherus cordiformis*, *Endamoeba ranarum*, *Trichomonas batracharum*, and *Hexamitus intestinalis*. Of 200 specimens of *Rana pipiens* from West Okoboji the incidence was as follows: *Opalina*, 99%, *Nyetotherus* 90%, *Endamoeba* 91%, *Trichomonas* 94%, *Hexamitus* 85%. Frogs from seven other localities showed similarly high percentages of infection except as follows: Diamond Lake, 5% *Nyetotherus*, 25% *Opalina*, 25% *Hexamitus*. Silver Lake, no *Nyetotherus*. Little Sioux River, no *Endamoeba*.

Pseudacris nigrita triceriata and *Acerisgryllus* from West Okoboji carried a fauna similar to that of *Rana pipiens*. In addition, however, *Pseudacris* was infected with *Endamoeba braziliensis* in the *Opalina* of 25% of the specimens.

Tadpoles of *Rana pipiens* from West Okoboji were infected as follows: *Opalina* 85%, *Nyetotherus* 100%, *Endamoeba* 36%, *Trichomonas* 40%, *Hexamitus* 98%. These protozoa were apparently completely lost during metamorphosis.

222. CHROMOSOME NUMBER IN LARVAE AND NEURAL-FOLD EMBRYOS OF *RANA FUSCA* DEVELOPED FROM PRICKED EGGS WITH KNOWN POLAR BODY AND EARLY CLEAVAGE HISTORY. Charles L. Parmenter, University of Pennsylvania.

The following individuals developed from eggs which gave off both polar bodies and were not delayed in early cleavages, and therefore would be expected to begin development with haploid nuclei. Three tadpoles had the expected haploid number. However, in an incomplete survey of another tadpole and of a neural fold embryo, mitoses containing approximately the triploid number (36 to 39) were present in the ectoderm, the wall of the neural tube, gut and muscles. Since both polar bodies

were extruded and there was no delay in early cleavage these triploid mitoses did not originate from the union of a retained polar body and a diploid group produced by a monaster in one of these early cleavages. Nor does it seem probable (although not impossible) that the second polar body, known to be given off, contained nothing but cytoplasm, having left its chromosomes in the egg to form a much delayed (at least 6 hours) union with a diploid nucleus produced by a monaster division or some other means in one of the blastomeres of the later cleavages.

223. MONO-, DI-, TRI- AND TETRA-NUCLEATE BLOOD CELLS IN PARTHENOGENETICALLY DEVELOPED *RANA FUSCA* TADPOLES. Charles L. Parmenter, University of Pennsylvania.

Blood cells containing one, two, three and four nuclei are present in the heart, capillaries and ventral region of the yolk mass of three haploid *Rana fusca* tadpoles (6 and 7 days old). The nuclei undergo mitosis and within a given cell are usually at the same stage at the same time. Whether each of these nuclei contains a full haploid set of chromosomes still remains to be determined. Cells with single haploid and with single diploid metaphases are also present, but triploids and tetraploids have not been seen. The blood cells of the normal tadpoles of approximately the same age so far examined contain only one nucleus.

224. THE OCCURRENCE AND DISTRIBUTION OF A THIRD TYPE OF GRANULAR CELL IN THE ANTERIOR PITUITARY OF THE CAT. Alden B. Dawson and Harry B. Friedgood, Harvard University and Harvard Medical School.

We have demonstrated (Friedgood and Dawson, '37) that the rabbit's anterior pituitary contains a third type of granular cell which is intensely and selectively stained with azocarmine after fixation in formalin-corrosive sublimate and staining with Heidenhain's azan modification of Mallory's method. A definite correlation has been established between the gonadotropic activity of the anterior pituitary and the presence of this cell. A similar but not morphologically identical cell has been found in the adult female cat. These cells in the cat tend to be located in the periphery of the gland. They are rather irregularly distributed throughout the postero-lateral region of the ventral one-third of the gland, forming a mosaic with the three regular classes of cells. In the medial antero-ventral region there is an irregular zone (zona tuberalis, Dawson, '37) in which basophiles and chromophobes predominate and few acidophiles are found. The special cells are concentrated in definite alveolar patterns about this zone and in the more dorsal postero-lateral regions. In these regions the cells appear pyramidal and granules are accumulated in the peripheral pole of the cell. These polar accumulations of granules are not so obvious in the scattered cells. These cells have not been found in male and female kittens and were rarely seen in the one adult male examined. An attempt is being made to correlate the changes in the number and character of these cells with the different phases of the reproductive cycle.

225. THE RATE OF MITOTIC ACTIVITY IN THE PITUITARY OF THE GROWING RAT. Gerard Roland Pomerat, Harvard University. (Introduced by Alden B. Dawson.)

Male and female white rats of various ages were injected with cholecicine and killed at the end of 8 hours. Sections of the pituitary gland were stained with Heidenhain's azan method and the number of mitoses calculated per square millimeter of tissue of uniform thickness. The total rate of mitotic activity in the pars distalis decreases regularly with increase in age and weight of the animal until at the end of the eighth week few mitoses can be found. The rate of activity in the pars intermedia, although somewhat lower, parallels that in the anterior lobe. Very few mitoses were seen in the pars nervosa even in young rats. Plotting separately the rates for each of the three cell types, that of the basophiles is lower but parallels the figures for the acidophiles, whereas mitotic activity in the chromophobe cells remains uniformly high during the first few weeks of life then falls more rapidly as age increases than do the other two. The effect of castration and of the different phases of the reproductive cycle on this comparative rate of mitosis in the various cell types is being studied but no conclusions have as yet been reached.

226. CYTOLOGICAL STUDY OF CHICK HEART MUSCLE IN TISSUE CULTURES. E. Frances Stillwell, Bryn Mawr College. (Introduced by D. H. Tennent.)

Tissue cultures from hearts of 8-day chick embryos were grown in a medium of fowl plasma and extracts of embryos of different ages (8 to 15 days). Early cultures (4 hours to 7 days cultivation) were fixed, sectioned and stained for cytological study. In toto mounts were made of cultures grown in vitro from 2 to 58 days.

Study of normal 8-day heart muscle (Helly, Zenker fixation or Champy-Kull process) shows that at this age differentiation is active. The striated myofibrils, when present, consist of simple light and dark bands. Fully differentiated myofibrils characterized by complex Q, J and Z bands are extremely rare. The cytoplasm of the 'pre-fibril' muscle cells contains an abundance of mitochondrial rods and granules.

Evidence is found in the early cultures of retrogressive changes which lead to the eventual degeneration of cross-striated muscle cells present at the time of explantation. Differentiation of the 'pre-fibril' muscle cells may occur almost at once or at a much later period.

227. THE GOLGI APPARATUS IN THE PROXIMAL AND DISTAL TUBULE CELLS OF THE PERFUSED FROG'S KIDNEY. Victor M. Emmel, Brown University. (Introduced by J. Walter Wilson.)

Frogs were perfused through the aorta with a pulsating flow of oxygenated Ringer's solution for periods of time varying from 30 minutes to about 9 hours. The Golgi apparatus was demonstrated by osmic impregnation according to Kolatchew's technique as modified by Nasonov. Within a half an hour after the beginning of perfusion the Golgi apparatus in both regions of the tubule becomes applied to the lateral surface of the nuclear membrane. This is followed by a period of fragmentation during which most of the fragments come to lie distal

or lateral to the nucleus, where they decrease in size and ultimately disappear. Disintegration of the Golgi apparatus occurs more rapidly in the proximal than in the distal tubules, and in the former is accompanied by the appearance within the cells of a substance which is diffusely darkened by osmic acid. The changes during perfusion differ in some respects from those occurring during autolysis in an excised kidney. The behavior of the Golgi apparatus during perfusion is in some respects comparable to its behavior in tissue culture. Disintegration during perfusion was not accompanied by the appearance of fatty droplets within the cells.

228. ABNORMALITIES INDUCED BY CENTRIFUGING FROG EGGS. H. W. Beams and R. L. King, State University of Iowa.

See abstract no. 131.

229. THE ABSORPTION OF COLLOIDAL CARBON FROM THE BODY CAVITY OF AMMOCOETES. Theodore W. Torrey, Indiana University.

Colloidal carbon injected into the coelom of the larval lamprey, *Ammocoetes*, is taken up directly by the pronephric tubules. Due to the absence of nephrostomes the mesonephric tubules do not function in a similar way. The tubules of neither show any intracellular deposition of carbon. The reticular elements which support both these kidneys exhibit pronounced phagocytic and hemocytopoietic activity. Carbon in either a free or included form reaches all the other organs both as a result of direct invasion or secondary distribution by the vascular system. The liver is the only organ whose vascular endothelium exhibits cytopoietic properties. To the diffuse spleen as a site of blood cell formation thus should be added the reticular tissue of the pronephros and mesonephros and the vascular endothelium of the liver. Playing a minor role in a similar way are the intestinal mucosa in addition to that in the typhlosole and the spongy tissue dorsal to the neural tube.

230. THE CILIARY GANGLION AND ASSOCIATED STRUCTURES IN THE GECKO, *GYMNODACTYLUS KOTSCHYI*. L. T. Evans and J. Minckler, Montana State University.

The usual eye-muscle nerve arrangement obtains in the gecko. The oculomotor supplies the superior, inferior and medial rectus muscles as well as the inferior oblique; the trochlear innervates the superior oblique; the abducens supplies the lateral rectus.

The ciliary ganglion of the gecko has two trunk roots: The oculomotor, assumed to convey motor fibers and the preganglionic parasympathetic fibers of the ganglion; the naso-ciliary, assumed to carry sensory fibers and post ganglionic sympathetic fibers contributed from a branch of the plexus of the carotid artery.

The eyeball of the gecko is innervated by three short ciliary nerves from the ciliary ganglion and by fibers which pass from the oculomotor to the sheath of the optic nerve. These fibers from the oculomotor are probably post-ganglionic parasympathetic.

The gecko appears to occupy a position midway in the evolutionary consideration of the ciliary ganglion, particularly with respect to the voluntary root and the distribution of fibers to the eye. The close relationship of the gecko to the bird is indicated throughout. This is to be expected since birds are supposedly derived from a reptilian source.

231. THE RELATION BETWEEN EYEBALL SIZE (RETINAL AREA) AND THE NUMBER OF FIBERS IN THE OPTIC NERVE OF THE DOG. Leslie B. Arey, Northwestern University Medical School.

Careful estimates of the total number of nerve fibers in the optic nerves of six variously sized dogs range from 137,681 to 162,243 (Arey, Castanares and Schaible).

A general correlation appears to exist between the size of the dog and the size of the eyeball, although the eyeball variation is a much more restricted and conservative phenomenon. Also these size variations apparently influence to a certain degree the numerical content of nerve fibers in the respective optic nerves.

The maximal areal variation encountered in the several retinas is as 100:170. This variation, however, does not affect proportionately the numerical total of optic nerve fibers, since the fiber variation is only 100:115.

232. CUTICULAR SCALES AND MEDULLA OF HAIRS FROM THE SEI WHALE (*BALAENOPTERA BOREALIS*). Leon A. Hausman, New Jersey College for Women (Rutgers University).

Among the whales, dolphins and porpoises, hair is present only very sparsely and is limited to areas about the muzzle. Eight hair-shafts from the Sei whale (*Balaenoptera borealis*) which had been examined in 1934 (Am. Nat., Jan.-Feb., 1934) showed only traces of cuticular scales and medulla. Two additional hair-shafts from this species of whale recently studied exhibited intact many complete cuticular scales, and several isolated patches of medullary columns. The scales were definitely of the flattened imbricate type, confirming the reconstruction which had previously been made. The medullary fragments were of the fragmental-medulla type, and several globular masses could be seen in some of the fragments. Both of these hair-shaft structures, therefore, conform to the types found correlated with hair-shafts of this diameter among mammals in general, and hence are not usable as criteria for the species of the group in general to which this whale belongs.

233. WEIGHTS AND LINEAR DIMENSIONS OF THE SKULL AND OF SOME OF THE LONG BONES OF THE RED-TAILED HAWK (*BUTEO BOREALIS BOREALIS*). Homer B. Latimer, University of Kansas.

This study is based on a collection of twenty-four male and twenty-seven female skeletons. The female skeletons are significantly greater in all of the weights; in nine of the fourteen skull dimensions and in all but two of the twenty-one linear dimensions of the long bones.

The coefficients of variation are somewhat greater in the males, or 3.8% for the weights, 1.6% for the fourteen skull dimensions and 6.6% for the twenty-one linear dimensions of the long bones. The skull weight is more constant in the females and the skull length in the males. In both sexes the skull weight is slightly less variable than the average, but in the males the three long bones of the wing are less variable and in the females the humerus and femur are less variable. The skull height is more constant in both sexes than the skull length. In the males, four of the skull dimensions are more constant than the skull length and in the females there are three dimensions less variable. In general the linear dimensions of the long bones are more variable than the dimensions of the skull.

The weights and linear dimensions of each member of the paired bone were obtained and the dominance of right or left bone are given in percentages of the total number of pairs studied.

Correlations of skull weight and skull length with the other weights and all of the linear dimensions are being prepared.

234. EXTERNAL MORPHOLOGY OF THE WHALE SHARK, *RHINEODON TYPUS*. L. Hussakof, American Museum of Natural History.

This study is based on a whale shark, 31.5 feet long taken by fishermen near Islip, on the south shore of Long Island, in August, 1935, and on numerous illustrations from photographs published by different authors. The object of the study was to clarify points incompletely known regarding this rare shark, and to prepare a correct figure of it.

The mouth is adapted for ingesting relatively small food, and is incapable of engulfing a small man, as has been stated. It is very wide, but the maximum vertical opening in a large shark is only 8 or 10 inches. The spiracle is not double, as stated in recent literature, but a single, small cleft, about $\frac{1}{2}$ inch, situated between the eye and the first gill.

The position and arrangement of the horizontal ridges on the sides, a peculiarity of this genus, are described. The lowermost ridge extends back to form the horizontal caudal keel. The peduncle is remarkably slender. A caudal dent is present at the upper origin of caudal.

Statements in the literature that the whale shark reaches 50 or 60 feet in length are unsupported by evidence. Recorded measured specimens indicate that it reaches a length of about 40 feet.

235. A COMPARISON OF THE NERVOUS SYSTEM IN NORMAL AND SINISTRAL SNAILS OF THE SPECIES *CAMPELOMA RUFUM*. Alice Savage, University of Illinois. (Introduced by Harley J. Van Cleave.)

Dextral individuals of *Campeloma rufum* have typical streptoneurous arrangement of the nervous system. Sinistral individuals show reversed symmetry of all organs posterior to the head region. Unpaired organs such as the gill, osphradium, kidney, heart and uterus occur on the opposite side of the body in sinistrals from where they occur in normal dextrals but the streptoneurous condition of the nervous system persists. For example, in dextrals nerves from the right ganglia innervate the single gill and osphradium which lie on the left side of the body while in sinistrals these same organs are on the right side of the body but are innervated by nerves originating in the ganglia of the left side.

236. REGENERATION OF THE GONAD TUBULES IN *THYONE BRIAREUS LESUEUR* FOLLOWING EXTIRPATION. Frank R. Kille, Swarthmore College.

Several accounts infer that the extensive powers of visceral regeneration in the *Holothuria* include the tufts of gonad tubules whenever these are lost in the process of autotomy. To test for this capacity, twelve male and twelve female *Thyone briareus* Lesueur collected in August were induced to autotomize the extreme anterior end of the body and the digestive system. The specimens were then

temporarily turned inside out in order to operate upon the gonad. In one group consisting of six male and six female individuals, only the tubules were removed. In the remainder, the entire gonad basis as well as the tubules was excised. Twenty-two of the specimens lived until killed for examination at various intervals between 30 and 68 days following the operation.

None of the second group gave any macroscopic indication of the proliferation of new tubules although restoration of the lantern and digestive system was complete at 22 days. On the other hand, each specimen in the first group proliferated one and usually two thick tufts of miniature tubules from the gonad basis. The new tubules were one-fifteenth the number and each one was approximately one-thousandth of the average size of the tubules which had been removed. Since only a small portion of the gonad basis is ever lost through the process of autotomy these results support the speculation that an eviscerated holothurian may proliferate a new set of gonad tubules.

237. FACTORS CONTROLLING REGENERATION IN TUBULARIA. L. G. Barth, Columbia University.

By dividing the size of the regenerating primordium of *Tubularia* by the time necessary for its formation a rate factor is obtained which serves as a measure of the rate of regeneration. The use of a ligature isolates the regenerating ends so that the independent rate of regeneration of the two cut ends may be measured. In other stems the relative rate of the two ends is obtained by measuring the rate of regeneration without a ligature. It is found that in long stems the relative rates of regeneration are directly proportional to the independent rates showing a partition effect. In short stems the relative rate of the distal (oral) end is greater than the independent rate, but the relative rate of the proximal (aboral) end is less than the independent rate.

This inhibition (dominance) was measured as the percentage reduction of the rate of regeneration of the proximal (aboral) end due to the presence of the distal primordium and was found to vary from 7% in long stems to 100% inhibition in short ones. This dominance exerted by the distal end could be blocked by injection of a drop of oil into the coelenteron stopping circulation.

238. PRODUCTION OF SUPERNUMERARY OUTGROWTHS IN PLANARIANS BY THE APPLICATION OF ETHYL ALCOHOL, ANILINE OIL AND COAL TAR. E. D. Goldsmith, College of the City of New York and Washington Square College, New York University.

Supernumerary head-like outgrowths have been produced in individuals of *Planaria maculata* by the application of either 80% alcohol, aniline oil or coal tar to the dorsal postcephalic and prepharyngeal regions.

A toothpick in which the tip was sharpened to a very fine point was dipped in the substance and the region to be treated was then lightly scratched with the tip. The epithelium was ruptured and the superficial parenchyma was exposed to the substance. The injured surfaces measured 0.3 to 0.7 mm.; those cases in which the injured surfaces were greater were discarded.

The supernumerary heads are similar in form to those produced by either radial incisions or micro-cautery (Goldsmith, Proc. Nat. Acad. Sci., 1934), and in a number of instances they possess more than the typical number of eyes. In some cases the outgrowths were resorbed; if these growths possessed eyes, the eyes remained as supernumerary ones.

In a number of the planarians it was necessary to apply the substances several times before outgrowths developed; in some, no growths developed after these applications.

The relative 'growth-producing' effectiveness of the alcohol, aniline oil and coal tar cannot be ascertained until experiments now in progress are completed. Additional experiments utilizing synthetic carcinogenic compounds are also in progress.

239. ANTERIOR REGENERATION IN THE ABSENCE OF CENTRAL NERVOUS TISSUES IN THE EARTHWORM, *EISENIA FOETIDA*. P. L. Bailey, Jr., College of the City of New York.

Ventral nerve cords in sixty-one individuals were transected at the fourth segment and the posterior pieces looped back at the eighth segment. Heads containing ganglia, commissures and cord were immediately amputated at segment 5 and the animals allowed to regenerate. Ten regenerated new heads with ganglia and cord continuous with the cord in the old part of the worm. Twenty-six healed without regenerating and showed histologically that the old cord remained looped. The others regenerated new heads each with ganglia, commissures and cord having no connection with the old cord, which remained looped back in every case. This group demonstrates conclusively that new nervous structures in anterior regeneration can arise from non-nervous elements without the influence of the old central nervous system. Earlier workers either removed part of the cord without looping, or, adapting the technique used by the present author in earlier work on posterior regeneration, looped the ends of the cord and allowed the wound to heal with an interval of several segments lacking the cord, later amputating the head in this region. The significance of the present experiments lies in the fact that the amputations were made at the same time as the operations for looping. Since the anterior nervous tissues were removed at the amputation and the posterior tissues remained looped back, nerve fibers which might be undetected histologically (especially fibers from the anterior pieces which are short and difficult to keep looped back) are prevented from regenerating across the gap during the interval before amputation.

240. ALTITUDINAL DISTRIBUTION OF FIREFLIES IN JAMAICA. John Bonner Buck, Johns Hopkins University.

During June and July, 1936, an extensive collection of fireflies was made in Jamaica, B.W.I. Although identifications are not complete, the following conclusions can be drawn.

1. A few species are apparently confined to a single, rather small district, but most species are fairly widely distributed, occurring in several localities at about the same altitude (same temperature and moisture).
2. In general each species is confined to a particular altitudinal range. Some, for example, are found only from sea level up to about 2500 feet; others extend from sea level to about 4500 feet; still others have an intermediate distribution, extending from about 3000 to 5000 feet. Several species are confined to the

higher mountains, some of them never having been found below 4000 feet, and still others, previously unknown, below about 7000 feet.

3. Certain species have a discontinuous distribution. One large mountain form, for example, is abundant on three different mountains at an altitude of 5000 feet, and wholly lacking in the intervening valleys.

241. LIGHT PENETRATION IN THE OKOBOJI LAKES, SUMMER 1937. Theodore L. Jahn and A. B. Taylor, State University of Iowa, University of Illinois and Iowa Lakeside Laboratory.

The penetration of visible light in the Okoboji lakes was measured by means of four photronic cells which were connected in parallel and submerged in a waterproof housing to various depths and a sensitive portable multi-range microammeter. The measurements were made on cloudless days, and the intensity measured by the submerged cells was recorded as a percentage of that measured by means of a surface cell which was covered by a neutral filter and maintained perpendicular to the sunlight.

In Lake West Okoboji 1% of the incident light penetrated to 7.0 meters, 0.1% to 10.7 meters, and 0.01% to 14.3 meters. Transmission of various 1-meter layers varied at different depths because of algal stratification. In Lake East Okoboji 1% of the light penetrated to only 0.9 meters and 0.01% to 1.95 meters.

These data show that West Lake might be tentatively classified as moderately turbid, and East Lake might be classified as extremely turbid.

242. A POINT IN THE RELATION BETWEEN CHARLES DARWIN AND THOMAS HUXLEY. Wm. E. Ritter, University of California.

Critical study of the utterances of these two men about morals shows them to have been rather far apart on the subject.

Darwin wrote in the *Descent of Man* that he agreed with those who consider the 'moral sense or conscience' to be by far the most important trait that differentiates Man from all the lower animals. But he also expressed the conviction that if this trait be examined from the side of natural history and in the light of evolution, as all other traits of all other animals are examined, the results correspond to the old familiar doctrine of the Golden Rule.

Huxley, on the other hand, ignored these views entirely, so far as known, and in his learned discussions of Evolution and Ethics developed his famous theory that "the cosmic process has no sort of relation to moral ends."

While this Huxleian view is not, I am sure, as somber as it has been widely felt to be, it certainly lacks much of being as roseate as the Darwinian view. But the real question is which contains the most truth? I believe it demonstrable that the Darwinian does; and believe further that had Huxley fully understood what Darwin meant by natural history; and had he adequately examined what Darwin understood by the 'moral sense,' his conclusions about Evolution and Ethics would have been very different from those he gave to the world.

243. THE GREATEST BIOLOGICAL PROBLEM. Alan Boyden, Rutgers University.

The greatest biological problem is, "What determines the characteristics of organisms?" A tentative outline of the solution of this problem is now available through the labors of many biologists. Briefly, there are two chief kinds of factors which determine the characteristics of organisms: 1) hereditary and 2) environmental. Each group of factors is composite, both are always present where characters are being determined, and the factors in each group react among themselves and with the factors of the other group in extremely complicated ways. Nevertheless, it is possible to estimate the relative importance, i.e., the relative effects of each kind of factor by holding one constant and varying the other. Altogether the results of observation and experiment justify these conclusions: 1) both heredity and environment are indispensable in the production of biological characteristics and both affect all kinds of characters, 2) the relative importance of any particular hereditary or environmental factor varies with the character and with the entire complex of hereditary and environmental factors concerned and can only be determined by observation and experiment, 3) neither hereditary nor environmental factors can fully compensate for defects in each other, 4) the nature of an organism is determined more largely by heredity than by environment. These principles provide the basic theory of phylogeny and of taxonomy and the practical basis for agriculture and eugenics. If they were untrue, phylogeny could not be determined and there would be neither biological perspective nor *natural* history in the world of life.

244. TERMITE NESTS—A STUDY OF THE PHYLOGENY OF BEHAVIOR. Alfred E. Emerson, The University of Chicago.

Termite nests may be used as examples of behavior evolution because they are morphological expressions of behavior patterns, they express the behavior of a population, the patterns are hereditary, there is a natural control over any Lamarckian influence, evolutionary sequences are available, adaptive modification may be demonstrated and coordination mechanisms may be partially analyzed. Concepts of homology and degenerative evolution may be applied to these behavior patterns.

Environmental stimuli affecting behavior response are mechanical factors, spatial factors, humidity, temperature, social factors, food and materials.

The nests may be excavated or constructed of dirt or wood cemented by saliva or anal excretions. Structures consist of covered tunnels, roadways, nests of characteristic size, shape, materials, organization and position. Ventilation pores, stored food and fungus gardens characterize certain groups.

The ecological function of the nest is the control of temperature, control of humidity, protection from predators and harmful fungi, all enabling the termites to live in otherwise uninhabitable niches. The nesting site is selected to some extent by the colonizing pair, but often is selected by the workers with a resulting colony migration.

Different species within a genus show divergence in nesting behavior. Species of the genus *Amitermes* have subterranean nests, mound nests, arboreal nests, nests oriented with reference to the sun, and rain-shedding constructions. Convergent evolution of rain-shedding constructions has occurred in the *Amitermitinae*, *Termitinae* and *Nasutitermitinae*.

245. ANYPHAENIDAE AND CLUBIONIDAE OF MICHIGAN. A. M. Chickering, Albion College.

The three species of Anyphaenidae and the twenty-two species of Clubionidae known to occur in Michigan are briefly described in this paper. Complete keys to the genera and species together with figures to illustrate the main diagnostic features are given. This is one of a series of papers on the spiders of Michigan.

246. DISTRIBUTION OF MAMMALS ON LAKE MICHIGAN ISLANDS. Robert T. Hatt, Cranbrook Institute of Science.

The indigenous mammalian faunas of North Manitou, South Fox and North Fox Islands, Lake Michigan, show complete uniformity. The species occurring were originally characteristic on the mainland where they are in part replaced by other species, invaders from the south since the clearing of the forests. No recent invader of the region has become established on the Islands except the house mouse in dwellings on North Manitou.

247. COPULATION IN THE ACOELOUS TURBELLARIAN POLYCHOERUS CARMELENSIS. Donald P. Costello and Helen M. Costello, University of North Carolina.

Copulation of acelous turbellaria has been recorded previously by Peebles for *Monchoerus lineatus* and by Hyman for *Amphiscolops langerhansi*. In *Polychoerus carmelensis*, the process, although of extremely brief duration, has been witnessed repeatedly in aquaria at the Hopkins Marine Station at Pacific Grove, California. Reciprocal copulation occurs in the following manner. Two restless individuals, upon meeting, elevate the anterior portions of their bodies, which sway slowly from side to side, and touch frequently or rub ventral surfaces. These individuals then twist sidewise and glide along each other in opposite directions, with ventral surfaces partly apposed and partly in contact with the substratum. The copulatory organs in the posterior fourth of the body, thus brought into juxtaposition, unite with a sudden snap. Thereupon one animal releases its attachment, the other remains attached by the posterior marginal papillae of one side, and both curl ventrally forming a ball, the typical copulatory position. Connection lasts 40 to 50 seconds and is terminated by uncurling and reattachment. Animals fixed during copulation invariably separated from each other. Sections reveal in the case of each individual that the penis is fully protruded and the vagina distended. A freshly deposited mass of sperm is usually found in the dorsal portion of the bursa.

248. EGG LAYING IN THE ACOELOUS TURBELLARIAN POLYCHOERUS CARMELENSIS. Donald P. Costello and Helen M. Costello, University of North Carolina.

There has been considerable speculation concerning the mode of egg laying among the aceles, with but few detailed observations. We have repeatedly observed *Polychoerus carmelensis* in the act of depositing eggs in finger-bowls at the Hopkins Marine Station. An individual, containing mature eggs, folds the anterior portion of the body ventrally while swaying slowly from side to side. Meanwhile a jelly-like slime is secreted from the body surface and adheres to the substratum upon contact. As the anterior portion continues its ventral curling,

the posterior end relinquishes its attachment, and folds ventrally under the approaching anterior tip. The worm thus forms a ball which is attached to the substratum by the secreted jelly. During this process violent muscular contractions are observable in the region of the vitellaria. The worm then begins to rotate slowly within its slime sheath, end over end. Meanwhile the eggs are being extruded from the body, but their point of emergence is completely hidden in the folds of the rotating animal. Rotation is terminated after 3 or 4 minutes, when the animal unfolds and works its way out of the slime sheath. The eggs remain in the central cavity of the jelly mass. Examination of serial sections of egg-laying worms indicates that the eggs are probably extruded through the ventral body wall rather than through either the mouth or the vagina.

249. A TREMATODE OF GENUS *MARITREMA* (NICOLL) PARASITIC IN THE BARNACLE AND THE RUDDY TURNSTONE. Charles E. Hadley and Ruth M. Castle, Montclair State Teachers' College and Vassar College.

Metacercarial and adult stages of a marine trematode, genus *Maritrema* have been found, respectively, in *Balanus balanoides* and in *Arenaria interpres*.

Spherical, yellow cysts measuring 0.136 mm. to 0.336 mm. were found in tissues surrounding the gut of *Balanus*. These cysts contained metacercariae measuring 0.576 mm. $\times$ 0.360 mm. with anterior and posterior suckers of approximately the same diameter, 0.055 mm.

The gut of an adult turnstone, killed while feeding on barnacle-covered rocks, contained adult flukes unmistakably of the same species as the above-mentioned metacercariae. The adults measured 0.858 mm. $\times$ 0.38 mm. with anterior and posterior suckers approximately equal, measuring 0.055 mm.; prepharynx and pharynx each 0.034 mm. in length; esophagus 0.153 mm.; gut diverticulae 0.239 mm.; testes 0.087 mm. $\times$ 0.112 mm.; ovary 0.068 $\times$ 0.146 mm.; eggs 0.0102 $\times$ 0.0204 mm. A well-developed seminal vesicle and cirrus sac is located immediately anterior to the acetabulum. The excretory vesicle is Y-shaped. The flame cell pattern is $2 \times (2 + 2) + (2 + 2)$. The vitellaria are confined to the posterior end of the body in circular pattern, turning mesially immediately anterior to the laterally located testes. The genital pore is at the left margin of the acetabulum and the ovary is just posterior to the acetabulum at the right. Gut diverticula extend to the level of the testes.

This fluke is certainly of the genus *Maritrema*. Its characteristics have not been found to correspond with those of any species as yet described and this trematode will probably be recognized as a new species.

250. THE FOOD OF PICKEREL. George W. Hunter, III, and J. Stewart Rankin, Wesleyan University and Amherst College.

Studies on the food of the banded pickerel (*Esox americanus*) and the chain pickerel (*E. niger*) as determined by stomach contents examinations show: 1) that the fish may be subdivided into two size groups according to their diet, one under 6 inches in length and one over; 2) that Crustacea and insects constitute the primary dietary item in the smaller group and consists of copepods, daphnia, crayfishes, chironomid larvae and pupae, mayfly and dragonfly nymphs, as well as considerable quantities of algae; 3) that the larger fish are essentially piscivorous, feeding on members of the Cyprinidae, Percidae, Centrarchidae, Etheostomidae, Esocidae, Ameiuridae, Gasterostomidae and Umbridae in decreasing order.

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NEW BOOKS AND PUBLICATIONS

SLEEP CHARACTERISTICS, How They Vary and React to Changing Conditions in the Group and the Individual, by N. Kleitman, F. J. Mullin, N. R. Cooperman and S. Titelbaum. 1937. Size, $6 \times 8\frac{1}{4}$ inches. Pages vii + 86. Bibliography. Cloth. Price, \$1.00. The University of Chicago Press, Chicago.

The investigations reported in this book were designed to learn what constitutes adequate sleep by finding the answers to the following three questions: 1) What are the differences and similarities between sleep characteristics of various individuals? 2) How uniform and constant are the values for the different sleep characteristics of a given individual. And 3) What are the influences of various external and internal conditions upon the characteristics or attributes of sleep? Records of thirty-six subjects were kept and the results tabulated. Such factors were considered as seasonal differences, ease of going to sleep, motility, duration, dreaming, and waking up well rested. Data collected have been treated statistically, results summarized into sixteen conclusions.

MOSAICS AND OTHER ANOMALIES AMONG ANTS, by William Morton Wheeler. 1937. Size, $6\frac{1}{2} \times 9\frac{1}{2}$ inches. 110 pages. 17 illustrations. Bibliography. Cloth. Price, \$2.00. Harvard University Press, Cambridge.

The ant colony on which the present study is based belongs to the fungus growing *Acromyrmex octospinosus*, and was collected in Trinidad. Among the population were numerous anomalous individuals, fifty-three of which are of unusual interest because, as the author explained in the preface, 'they are quite unlike any previously observed among ants or indeed any other social insects; and they enable us to decide between two theories of caste determination which have baffled and divided the students of ants for more than half a century.' The distribution, habits and normal castes of *Acromyrmex* are described as a background for a close study of the anomalies of *Acromyrmex octospinosus*. The last chapter gives facts and inferences bearing on the controversy as to whether the dimorphism of the female sex is due merely to differential larval feeding or is already predetermined in the eggs from which they develop. In two appendices are added taxonomic notes on *Acromyrmex octospinosus*, and a revision of the known non-mosaic female and worker anomalies of ants.

PRACTICAL NEUROANATOMY, A Textbook and Guide for the Study of the Form and Structure of the Nervous System. Adapted to the needs of the student and practising physician, by J. H. Globus. 1937. Size, $8 \times 10\frac{1}{4}$ inches. Pages xxii + 387. 92 illustrations and 12 plates. Index. Fabrikoid. Price, \$6.00. William Wood and Company, Baltimore.

The structure of the central nervous system is sometimes considered to be the most intricate and difficult branch of anatomy. The author of this book feels that such a belief is unjustified if the student can be made to realize that the central nervous system has a fairly uniform architecture consisting of little more than a series of nuclei and tracts. Surface anatomy supplies the landmarks by which the nuclei and tracts can be recognized. The subject matter in this book is divided into descriptive text and assignments, each assignment being planned to provide work for a 2-hour laboratory period. Plates printed with outline drawings of different parts of the brain are supplied on which the student can draw and label the structures found and identified by dissection. The book also contains a number of clinical examples of disease involving the brain

NEW BOOKS AND PUBLICATIONS

and spinal cord. Another useful feature is the final chapter which gives methods of preparing, preserving and staining nervous tissues, together with a brief history of their discovery, development and use.

DIATHERMY, Including Diathermotherapy and Other Forms of Medical and Surgical Electrothermic treatment, by Elkin P. Cumberbatch, with nine collaborators. Third edition. 1937. Size, $6 \times 8\frac{1}{4}$ inches. Pages xvi + 576. Index. 169 illustrations. Cloth. Price, \$6.00. William Wood and Company, Baltimore.

The title 'Diathermy' has been retained in the third edition although the book has been enlarged to include all forms of treatment conducted by means of currents that oscillate with high frequency. Part I of the book, introductory in nature, is concerned with the history of diathermotherapy and its physical and electro-technical principles. Part II treats of medical electrothermic methods and their uses. It distinguishes diathermy and diathermotherapy from effluviation and étincelage, short wave treatment and inductothermy. Part III explains the respective surgical uses of electrodisection, electrocoagulation and electrosection.

PHYSIOLOGY FOR PHARMACEUTICAL STUDENTS, by Harold Hayden Barber. 1937. Size, $6 \times 8\frac{1}{4}$ inches. Pages viii + 478. 132 illustrations. Index. Cloth. Price, \$4.50. William Wood and Company, Baltimore.

This textbook of physiology is planned specifically for pharmaceutical students. Very little anatomy is included, only the bare requirements necessary to proper understanding of the bodily functions. Histology, experimental physiology and physiological chemistry are studied in more detail with appropriate laboratory exercises to familiarize the student with practical application of principles and technique. Following the discussion of blood and its properties, for instance, the laboratory experiments show methods for collecting blood samples, red and white cell counts, blood grouping, analyses for hemoglobin, oxygen, carbon dioxide and other gases. A chapter on pharmacology of typical substances is divided into drugs which act on the alimentary canal, drugs which act on the nervous system, etc. The final chapter describes the preparation and standardization of reagents and staining solutions.

DIE DOPPELBRECHUNG VON KARYOPLASMA, ZYTOPLASMA UND METAPLASMA, by W. J. Schmidt. 1937. $6 \times 8\frac{1}{4}$ inches. Pages xi + 388. Bibliography. Index. 89 illustrations. Cloth. Price, RM 24. Gebrüder Borntraeger Verbuchhandlung, Berlin.

This is the eleventh volume of a series 'Protoplasma Monographien' dealing with the properties of protoplasm. It investigates the phenomena of double refraction in living substance: its nature, appearance and causes in nuclei, cytoplasm and the specialized structures of various kinds of tissue.

GRUNDRISS DER ENTWICKLUNG DES MENSCHEN, by Alfred Fischel. Second edition. 1937. Size, $6\frac{1}{4} \times 10$ inches. Pages v + 143. 117 illustrations. Index. Cloth. Price, RM 12.60. Julius Springer, Berlin.

The first section of this book treats of germ cell origin and fertilization. The next part takes up early embryonic stages, development of body form, placentation, foetal membranes; and the rest of the book is devoted to growth of body organs and systems in the foetus.

SUPPLEMENT

AMERICAN SOCIETY OF ZOÖLOGISTS

Officers for 1938

<i>President</i>	M. H. JACOBS
<i>Vice-President</i>	T. S. PAINTER
<i>Secretary</i>	E. G. BUTLER
<i>Treasurer</i>	WALTER N. HESS

Executive Committee

(In addition to the four officers)

CHARLES ZELNY.....	to serve until December, 1938
A. H. STURTEVANT.....	to serve until December, 1939
ROBERT W. HEGNER.....	to serve until December, 1940
W. C. ALLEE.....	to serve until December, 1941
F. L. HISAW.....	to serve until December, 1942

*Representative of the Society in the Division of Biology and Agriculture of the
National Research Council*

To serve until July 1, 1941.....	LAURENCE IRVING
	A. C. REDFIELD, Alternate

*Representatives of the Society on the Council of the American Association for the
Advancement of Science*

H. V. NEAL

A. A. SCHAEFFER

*Representatives of the Society on the Council of the Union of American
Biological Societies*

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L. V. HEILBRUNN.....	<i>General Physiology</i>

*Representative on the Executive Committee of the Commission for the
Standardization of Biological Stains*

S. I. KORNHAUSER

Managing Editor of the Journal of Morphology

To serve until December, 1941.....	C. E. MCCLUNG
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Members of Editorial Board of the Journal of Morphology

To serve until December, 1938

SALLY HUGHES-SCHRADER	E. E. JUST	D. H. WENRICH
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To serve until December, 1939

LEIGH HOADLEY	W. A. KEPNER	J. PERCY MOORE
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To serve until December, 1940

J. H. BODINE	N. E. MCINDOO	H. H. PLOUGH
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PROCEEDINGS OF THE THIRTY-FIFTH ANNUAL MEETING OF THE AMERICAN SOCIETY OF ZOÖLOGISTS

INDIANAPOLIS, INDIANA, DECEMBER 28, 29, 30, 1937

PROGRAM

The Society met in conjunction with Section F of the American Association for the Advancement of Science and in association with other biological societies. All scientific sessions were held at the Indiana University School of Medicine in Indianapolis.

In addition to the titles and abstracts printed in the December supplement to *The Anatomical Record*, the Society by vote at the business meeting authorized the printing of the following late title and abstract:

251. THE EMPLOYMENT OF ROENTGEN RADIATION IN THE STUDY OF EMBRYONIC DEVELOPMENT. Titus C. Evans, A. and M. College of Texas.

X-rays may be used in experimental embryology because of their selective action. It is possible to alter developmental processes in such ways as to support evidences gained by other methods and to disclose some properties hitherto unknown.

The material used was the egg of *M. differentialis*. The method was to irradiate eggs of different ages with varying amounts of radiation and to follow the subsequent morphological and physiological changes.

It was found that the eggs became more resistant as the embryos became larger and more differentiated. The stage of development during which the embryo was first being outlined was an especially susceptible one. Embryos rayed at this time developed abnormal growths which in advanced cases appeared to be attempts to form secondary embryos. The morphological studies seem to support ideas of axial gradients and organization centers.

Certain experiments on the effects of radiation on the rate of oxygen consumption have resulted in some ideas as to the relation of oxygen consumption rate to embryonic activity.

The Indianapolis meeting was one of the largest meetings of the Society ever held. The total number of papers on the program to be read was 130, to be presented by demonstration, 31, to be read by title, 95. (Included in the foregoing figures are papers in symposia and special programs, as well as the late title recorded above.)

The Biologists' Smoker was held on Tuesday evening, December 28th, in the Riley Room of the Claypool Hotel, with an attendance of approximately 800 people. The annual dinner of the Society was held in the same room on Wednesday evening, December 29th, with 246 persons attending. Prof. Ralph S. Lillie, vice-president of Section F of the A.A.A.S. gave an address on "The Nature of Organizing Action."

BUSINESS MEETING

The thirty-fifth annual business meeting opened at 12.10 P.M. December 29th, President F. L. Hisaw presiding.

The following business was transacted:

1. The minutes of the last meeting, having been printed in January, 1937, supplement to *The Anatomical Record* and distributed to all members of the Society, were approved as published.

2. By vote of the Society the secretary was authorized to publish a late title and abstract in the January, 1938, supplement to *The Anatomical Record*.

3. *Report of the secretary.*

a. Membership. During the last year the membership of the Society has undergone the following changes (including election of new members later in the meeting):

Deaths	8	Reinstated	5
Resignations	4	New active members	59
Dropped	8	New associate members	54
Total enrollment at present		827	
Total enrollment in last report		767	
Gain in enrollment		60	

Summary of enrollment

Life members	5
Active members	629
Associate members	193
Grand total	827

b. At the last annual meeting it was voted to designate the charter members of the American Morphological Society on the list of present members of the Society. This will be done in the publication of the 1938 membership list. For the information of the Society the secretary reported as follows: There were 26 charter members of the American Morphological Society, which was organized in 1890. Of this number 10 are still living, and 6 are at present members of the American Society of Zoölogists.

It was voted to accept the report of the secretary.

4. During the last year the Society has suffered the loss by death of eight active members: Frank Nelson Blanchard, Lucy Smith Clemens, Henry Homer Collins, William Harding Longley, Hansford M. MacCurdy, Hugh Daniel Reed, Will Scott and William Morton Wheeler. The following resolutions were adopted by a rising vote of the Society, and the secretary was instructed to send copies to the nearest relatives and to publish the resolutions in the proceedings of the Society.

FRANK NELSON BLANCHARD

Dr. Frank N. Blanchard died in Ann Arbor, Michigan, September 21, 1937, from a streptococcic infection. He was born December 19, 1888, at Stoneham, Massachusetts. After graduating in 1913 from Tufts College he taught zoölogy for three years at Massachusetts Agricultural College. Then he became a student of Alexander G. Ruthven at the University of Michigan, where he secured the degree of doctor of philosophy. From 1918 to 1919 he served in the Division of Reptiles of the United States National Museum. In 1919 he joined the staff of the Department of Zoölogy of the University of Michigan, where he held the rank of associate professor at the time of his death. Doctor Blanchard was eminent for his studies on the natural history and classification of reptiles and amphibians and he had published many papers on these classes of animals.

L. R. DICE.

LUCY SMITH CLEMENS

Lucy Smith Clemens was born in Scheffield, Massachusetts, January 3, 1887. She graduated from Mount Holyoke College in 1909. Later she studied at Cornell University, receiving the A.M. degree in 1911 and the Ph.D. degree in 1914. Doctor Clemens was successively research assistant at the station for the study of experimental evolution of the Carnegie Institution at Cold Spring Harbor, assistant in biology at Cornell University and instructor in zoölogy at Mount Holyoke College. Later she served as assistant director of the Pacific Biological Station at Nanaimo, British Columbia, of which her husband, Dr. Wilbert A. Clemens is director.

Her investigations and publications were concerned primarily with fresh water and marine animals, including aquatic insects, turtles and fishes. Doctor Clemens died at Nanaimo, British Columbia, on May 23, 1937.

ANN H. MORGAN.

HENRY HOMER COLLINS

Henry Homer Collins, professor of biology, University of Pittsburgh, was born in Kewanee, Indiana, December 10, 1885. The University of California granted him the B.S. degree in 1915, the M.A. in 1916 and the Ph.D. in 1919. He has been successively an instructor of history in the Rochester Normal School; superintendent of schools at Pilomath 1908 to 1910 and at Alsea in 1910 to 1913; a teaching fellow in zoölogy at the University of California, 1914-1915; LeCante fellow, 1915-1916, research assistant at the Scripps Institute, 1916-1919. He later served as head of the department of biology in the high school and junior college at Fresno, California, in 1919 to 1920. In 1920 he went to the University of Pittsburgh as assistant professor of biology and in 1925 was promoted to full professorial rank. He died on August 31, 1937, after an illness of six months. His wife, two sons and a daughter survive him. He was a member of the American Association for the Advancement of Science, the American Society of Zoölogists, the Society of Mammalogists and Sigma Xi. His research interests have been centered primarily on problems of growth as exhibited in regeneration phenomena in salamanders. In this field he was the author and co-author with his students of a number of papers.

ROBERT T. HANCE.

WILLIAM HARDING LONGLEY

William Harding Longley, professor of biology and director of the marine biological laboratory at the Tortugas Islands, died on March 10, 1937. He was born in Paradise, Nova Scotia, October 27, 1881, and graduated from Acadia University in 1898. He then accepted a principalship in the public schools of Nova Scotia and held it for five years, after which he entered Yale University and received the degree of B.A. in 1907, M.A. in 1908 and Ph.D. in 1910. He was an instructor in Yale University for one year and then became instructor in biology and associate professor of botany in Goucher College, where he was advanced to the rank of professor of botany in 1914 and professor of biology in 1919.

In 1931 Acadia University conferred upon him the honorary degree of doctor of science. He was a member of Phi Beta Kappa, Sigma Xi, the American Society of Zoölogists and various other scientific societies.

Shortly after Doctor Longley became connected with Goucher College he accepted the position of collector at the Tortugas laboratory and with this laboratory he remained closely associated as investigator and director until his death. Here he made extensive observations on the tropical reef fishes, particularly in reference to protective coloration and its bearing on the problem of evolution. In making these observations he initiated the use of a diving helmet and spent much time under water in close association with the fishes in their natural habitat. Similar observations were also made on the reef fishes in Hawaii, Samoa and the Dutch

East Indies. He also made a thorough study of type specimens of reef fishes in all the more important museums. He thus devoted years of study to the reef fishes the world over and came to know them and their habits in relation to their environment far more thoroughly than any one else had ever known them. The results of this study are presented in excellent form in an extensive monograph, richly illustrated with marvelous photographs and drawings of reef fishes under various conditions in their natural habitats. This monograph was so nearly finished at the time of his death that it will doubtless be published. All this work was amply supported by the Carnegie Institution of Washington.

Doctor Longley was a far sighted, ingenious investigator, a very stimulating teacher and a true friend. The American Society of Zoölogists has lost a most substantial member.

S. O. MAST.

HANSFORD M. MACCURDY

Hansford M. MacCurdy was born in Warrensburg, Missouri, on October 3, 1867. After receiving elementary schooling in Warrensburg he prepared for teaching at the Central State Teachers' College in the same community. In 1895 he was graduated from the Ohio Wesleyan University. Four years spent as superintendent of schools at Mt. Vernon were followed by five years of service in the Manual Training High School at Kansas City, Missouri. In 1904 he took up advanced studies at Harvard University, where he was granted the degree of doctor of philosophy in 1906. He was immediately called to Alma College to act as head of the department of biology, a position which he occupied until his death on May 24, 1937.

Professor MacCurdy's special investigations were in the field of heredity, one of his early papers dealing with the inheritance of coat pigments and coat patterns in rats and guinea pigs, and a later one with nuclear reorganization in relation to conjugation and inheritance in *Arcella*. At frequent intervals he carried on researches at Harvard University, The Johns Hopkins University, Woods Hole, Cold Spring Harbor and Douglas Lake.

In spite of his continuous effort to contribute to knowledge in his special field in the few hours left to him from a heavy teaching schedule, Professor MacCurdy maintained throughout his life the deepest interest in advances in other branches of natural science. Perhaps his most lasting contribution was made through his devotion to his many students, who derived enduring inspiration from his breadth of view, his untiring enthusiasm, his keen powers of observation, his awareness of the significance of the things observed, his delightful sense of humor and his example as a cultured gentleman.

Professor MacCurdy is survived by his wife, two sons, three daughters and one brother, Prof. George G. MacCurdy.

L. W. SHARP.

HUGH DANIEL REED

Prof. Hugh Daniel Reed was born at Huntsville, New York, on March 4, 1875. He completed his undergraduate course of study in Cornell University in 1899, and on being appointed a fellow in vertebrate zoölogy, he continued there in graduate study for four years, obtaining his doctor's degree in 1903. He taught at Cornell under the direction of Dr. Burt G. Wilder successively as assistant, instructor and assistant professor from 1900 to 1910. Then he spent a year on leave of absence in Germany at Freiburg, working with Weismann, Wiedersheim and Gaupp.

He returned to Cornell as head of the department of vertebrate zoölogy after Doctor Wilder's retirement, and continued in active charge until the time of his death in Ithaca on August 23, 1937. He was an excellent organizer, and developed the courses in his department with balance and proportion, and provided equipment for their conduct with practical judgment and foresight. He organized his own lectures in the same careful way, and they were models of clearness, and conciseness. Although these duties mainly absorbed his time and energy he never abandoned research.

Within his department Doctor Reed was a constant source of advice and inspiration. Those who were associated with him will not soon forget the debt they owe him as counselor and friend. As a token of their affection for him his pupils presented an oil portrait of him to the university in 1934.

During years of continuing ill health he carried on with unabated zeal. His many personal friends will deeply regret the loss of a man in whom personal charm, wide culture, and balanced judgment were combined to an unusual degree.

JAMES G. NEEDHAM.

WILL SCOTT

Dr. Will Scott was born at Houston, Indiana, April 20, 1877, and died October 17, 1937. His early training was in the grade and high schools of the state. This was followed by work at the Indiana State Teachers College at Terre Haute, Indiana, and at Indiana University where he graduated in 1908. He was granted the Ph.D. degree in the latter institution in 1911. His teaching career began at Indiana in 1908 and continued there until his death. Since 1920 he had been director of the Biological Station at Winona Lake in Northern Indiana.

His researches were in the field of limnology and while the number of his papers was not large, his knowledge of the subject was profound. In the opinion of the writer it is doubtful whether he was surpassed by anyone with respect to his breadth of view and his grasp of the whole field of freshwater biological problems.

Doctor Scott's sympathetic and understanding attitude toward students made him an excellent teacher, particularly when working with individuals or small groups of students.

Toward his colleagues and others he was always congenial, friendly, cooperative, unselfish. He gave all he had to his university and to society. What more can any man do?

FERNANDUS PAYNE.

WILLIAM MORTON WHEELER

William Morton Wheeler, one of the most distinguished of American entomologists, was born in Milwaukee, Wisconsin, March 19, 1865, and died suddenly in Cambridge, Massachusetts, April 19, 1937. Professor Wheeler's long career as investigator, administrator, and teacher extended well over fifty years. During this period he held offices of distinction and responsibility in the Public Museum of Milwaukee, Clark University, The University of Chicago, the University of Texas, the American Museum of Natural History and finally Harvard University. Here he spent the last thirty years of his life and here the unusual promise of his early youth reached full fruition. His scientific interests were in the beginning directed toward the insects and, though his subsequent studies led him in many other directions, this group of animals retained his lasting attention. Of the 300 and more scientific publications that came from his pen, the majority were entomological and most of these had to do with ants. His study of these minute creatures touched every side of their lives and his investigations into their social habits led him to reflect and write on insect societies in contrast with human civilization. This subject aroused him to some of his rarest efforts where, with his unusual literary ability and his keen sense of humor, he exposed the failings of his own species. Professor Wheeler's genius was universally recognized and he was the recipient of many honors and distinctions. He was a charter member of this body when it was known as the American Morphological Society and after it changed its designation to the American Society of Zoölogists, he served it in 1908 as one of its presidents.

G. H. PARKER.

5. Report of the treasurer.

The treasurer presented the following report:

*Report of the treasurer for the year 1937**Credits:*

Balance on hand December 28, 1936, when account was last audited	\$1332.28	
Receipts from dues, 1937	2798.00	
From H. B. Goodrich, secretary	25.00	\$4155.28

Expenditures:

Naturalists' smoker, Atlantic City	15.00	
Zoölogists' dinner, Atlantic City	21.20	
F. Payne, conference expenses	2.50	
W. C. Curtis, program expenses	3.73	\$42.43

Secretary's expenses:

Stenographer	67.36	
Printing and stationary	76.54	
Postage and express	29.31	
H. B. Goodrich, secretary, expenses	49.29	
E. G. Butler, stipend	200.00	422.50

Treasurer's expenses:			
Postage	33.00		
Secretary	54.02		
Printing	26.94		
Miscellaneous expenses	1.76	115.72	
Wistar Institute:			
Sale of abstracts, Atlantic City	25.00		
Proceedings and list of members	168.18		
Abstracts	498.93		
One line cut	4.34	696.45	
Biological abstracts		1228.00	
Bank charges		15.14	
		2520.24	
Balance, December 27, 1937:			
Checking account	635.04		
Savings account	1000.00	1635.04	
		\$4155.28	\$4155.28
Increase in funds since December 22, 1936, amounts to \$302.76			

6. *Report of the auditing committee* (C. L. Turner and L. E. Noland).

Dr. C. L. Turner, chairman, reported that the accounts of the treasurer had been examined and found to be correct.

It was voted to accept and approve the reports of the treasurer and of the auditing committee.

7. *Report of the representative on the executive committee of the Commission for the Standardization of Biological Stains.*

Dr. S. I. Kornhauser presented the following report, which was approved:

As representative of the zoölogists on the Committee for the Standardization of Biological Stains, I wish to report that during the past year, the high standards of American biological stains has been maintained through our testing system and certification. Your representative has tested biologically all samples submitted to him by the chairman of the commission and reported promptly to him results obtained.

The third edition of Biological Stains appeared during the past year. It is enlarged by the addition of fifty pages. It has received warm recognition from biologists as evinced by its sale.

Stain Technology also has been well received and its circulation increased. Its publication of abstracts on microscopic technique has been greatly enlarged. The abstracts are written with object of giving sufficient information to the worker to follow the technical procedures described in the original papers. Your representative has contributed abstracts from three journals assigned to him.

The members of the zoölogists are urged again to communicate with your representative if you find any certified stains unsatisfactory or if you desire additional dyes put on the certification list. We should also be glad to have technical papers submitted for publication in Stain Technology.

8. Report of committee on educational testing.

Dr. D. E. Minnich, chairman, presented the following report:

Several years ago this society appointed a committee consisting of W. C. Allee, R. A. Budington and D. E. Minnich to study the question of objective examinations for courses in elementary zoölogy of collegiate grade in cooperation with the test service of the American Council of Education. For the past 2 years your committee has given consideration to this question and now submits the following report:

1. The committee is not clear that objective tests will find any interest or following on the part of more than a small minority of the zoölogy teachers in zoölogy departments in universities and colleges.

2. The committee is confessedly unwilling to adopt the test sheets sent it by the Cooperative Test Service.

3. The committee is unwilling to recommend to the Society of Zoölogists any plan for endorsement until some canvass of an appreciable number of the membership has been made. The committee has neither the means nor the time for conducting such a survey.

Under these conditions the committee finds itself unwilling to endorse or sponsor the proposed test program and requests the society for its discharge.

It was voted to accept the report of the committee and place it on file.

9. Election of new members.

In accordance with the stipulations of the constitution the executive committee recommended the following candidates for election to membership in the society:

ACTIVE MEMBERS

(Formerly associate members)

Abramovitz, Alexander A.	Gordon, Albert Saul	Rosene, Hilda F.
Bevelander, Gerrit	Graham, George Laurin	Schweizer, Malvina
Branch, Hazel Elizabeth	Gray, James Clarke	Sweetman, Harvey Leroy
Brauer, Alfred	Greep, Roy Orval	Trager, William
Browman, Ludvig Gustav	Hall, Edmund Kennard	Tressler, Willis Lattanner
Brown, Frank Arthur, Jr.	Hellbaum, Arthur Alfred	Warren, Arthur Emerson
Buchsbaum, Ralph	Kopac, Milan James	Wells, Lemen Jonathan
Buck, John Bonner	Korr, Irvin Morris	Wichterman, Ralph
Child, George Percy	Loefer, John B.	Wilson, Francis Henry
Cloudman, Arthur Mosher	Loosanoff, Victor L.	Wilson, Ira Templin
Crescitelli, Frederick	Lumer, Hyman	Wolfe, Harold Reclus
Dalton, Albert J.	Pomerat, Charles Marc	Woodside, Gilbert Llewellyn
Geiman, Quentin Monroe	Root, Raymond Willard	

ACTIVE MEMBERS

(Not formerly associate members)

Bozler, Emil	Johnson, Frank Harris
Burger, J. Wendell	Kaston, Benjamin Julian
Byrd, Elon E.	Kaufmann, Berwind Petersen
Casida, Lester Earl	Kempton, Rudolf T.
Clemens, Wilbert Amie	Oppenheimer, Jane M.
Dempster, Wilfrid Taylor	Poulson, Donald Frederick
Elliott, Rush	Schmidt, Karl Patterson
Goldsmith, Joseph Benjamin	Schooley, James Plummer
Haig, Charles	Taylor, Aubrey Bryant
Haskins, Caryl Parker	Yeager, J. Franklin
Hull, Frank Montgomery	

ASSOCIATE MEMBERS

Alexander, Lloyd Ephraim	Johnson, David F.
Anderson, Marlowe George	Jones, Roy Winfield
Angerer, Clifford Ackermann	Kleinholz, Lewis Herman
Ashkenaz, Eleanor Weiner	Lane, Charles Edward
Bailey, Sarah Watson	Litwiller, Raymond
Berger, Charles A.	Lovell, Harvey Bulfinch
Blount, Isabel Harper	Mattox, Norman T.
Bradbury, James T.	Mazia, Daniel
Bragg, Arthur Norris	Melampy, Robert M.
Bunde, Carl A.	Milne, Lorus Johnson
Burdick, Harold Ormond	Moritz, Charles Edgar
Bushnell, Ralph Judson	Neave, Ferris
Carpenter, John Richard	Quiring, Daniel Paul
Carroll, Paul L.	Robinson, True William
Corey, H. Irene	Rogers, Philip V.
Daugherty, Kathryn	Root, Clinton W.
Dempsey, Edward Wheeler	Ruebush, Trenton Kieffer
Derrick, Grace Ethel	Self, John Teague
Dornfeld, Ernst John	Shettles, Landrum Brewer
Eastlick, Herbert Leonard	Shlaer, Simon
Eaton, Orson Northrop	Sizer, Irwin Whiting
Engels, William Louis	Spoor, William Arthur
Fuller, John Langworthy	Strohecker, Henry Fred
Gatz, Arthur John	Taylor, Alfred
Hasler, Arthur D.	Turner, Clarence Donnell
Hefley, Harold Martin	Van Horn, Willis M.
Howell, Charles DeWitt	Welch, d'Alte Aldridge

It was voted that all candidates be elected as recommended by the executive committee and the secretary was instructed to cast one ballot in their favor.

10. *Report of executive committee.*

The executive committee presented the following report and recommendations:

a. In accordance with a motion made at the 1936 annual meeting, the committee has studied the problem of making provision for honorary membership in the Society. Such provision will require an amendment to the constitution. The committee made a report of progress and placed in the hands of members a tentative proposal in regard to a constitutional amendment. Members who wish to make suggestions regarding this proposal were requested to do so in

writing to the secretary before June 1, 1938. The committee recommended that definite action on a constitutional amendment concerning honorary membership be taken at the 1938 annual meeting.

b. The committee recommended that next year the final date for submitting titles and abstracts to the secretary be November 1. This recommendation was made because of the great increase during recent years in the number of papers on the program and the consequent necessity for more time in arranging the various sections.

c. The committee recommended that \$50.00 be appropriated toward the expenses of the Third International Congress for Microbiology to be held in New York City during the summer of 1939.

d. The committee recommended the support of Biological Abstracts for 1938 on the same plan as for 1937, namely, that \$2.00 from each membership fee of \$4.00 be paid to Biological Abstracts.

It was voted to approve the report of the executive committee and accept the recommendations.

11. *Report of nominating committee and election of officers.*

The nominating committee, consisting of Caswell Grave, chairman; P. W. Whiting; C. V. Taylor; presented the following report:

President, 1 year: M. H. JACOBS.

Vice-president, 1 year: T. S. PAINTER.

Executive committeeman, 5 years: F. L. HISAW.

Society representative, Division of Biology and Agriculture, National Research Council, 3 years: LAURENCE IRVING; A. C. REDFIELD, Alternate.

Society representatives on Council of A.A.A.S., 1 year: A. A. Schaeffer, H. V. Neal.

Society representatives on Council of Union of American Biological Societies, 1 year: SEWALL WRIGHT, A. F. SHULL.

Society representatives on Advisory Editorial Board of Biological Abstracts: H. S. PRATT, G. K. NOBLE, D. H. TENNENT, L. V. HEILBRUNN.

Representative on the executive committee of the Commission for the Standardization of Biological Stains: S. I. KORNHAUSER.

Members of editorial board of the Journal of Morphology, 3 years: H. H. PLOUGH, J. H. BODINE, N. E. MCINDOO.

It was voted that all candidates be elected as recommended by the nominating committee and the secretary was instructed to cast one ballot in their favor.

13. The president announced his appointment of the nominating committee for 1938 as follows: G. H. Parker, chairman; W. C. Curtis; Fernandus Payne.

14. The following resolution, presented by Dr. D. E. Minnich, was unanimously approved:

Resolved that the American Society of Zoölogists through the secretary send to the chairman of the Local Committee for the Indianapolis Meeting a formal expression of sincere thanks for the efficient arrangement and cordial hospitality provided by the committee for the society at its Indianapolis meeting.

The meeting was adjourned.

E. G. BUTLER,
Secretary.

LIFE AND ACTIVE MEMBERS

(* indicates life members of the Society; † indicates charter members of the American Morphological Society; mail addresses are in italics.)

- ABRAMOWITZ, ALEXANDER A., B.A. (St. Stephen's), M.A., Ph.D. (Harvard), Research Assistant, *Biological Laboratories, Harvard University, Cambridge, Mass.*
- ACKERT, JAMES EDWARD, A.B., A.M., Ph.D. (Illinois), Professor of Zoölogy, Parasitologist, Agr. Exp. Station; Dean, Division of Graduate Study; *Kansas State College, Manhattan, Kan.*
- ADAMS, A. ELIZABETH, B.A. (Mt. Holyoke), M.A. (Columbia), Ph.D. (Yale), Professor of Zoölogy, *Mount Holyoke College, South Hadley, Mass.*
- ADAMS, LEVERETT ALLEN, A.B., A.M. (Kansas), Ph.D. (Columbia), Assistant Professor of Zoölogy, University of Illinois, *406 Nevada St., Urbana, Illinois.*
- ADAMSTONE, FRANK BOLTON, B.A., M.A., Ph.D. (Toronto), Associate in Zoölogy, *University of Illinois, Urbana, Ill.*
- ADELMANN, HOWARD BERNHARDT, A.B., A.M., Ph.D. (Cornell), Professor of Histology and Embryology, *Stimson Hall, Cornell University, Ithaca, N. Y.*
- ADOLPH, EDWARD FREDERICK, A.B., Ph.D. (Harvard), Associate Professor of Physiology, School of Medicine, *University of Rochester, Rochester, N. Y.*
- AGERSBERG, H(ELMER) P(ARELI VON WOLD) K(JERSCHOW), B.S., M.S. (Washington), M. A. (Columbia), Ph.D. (Illinois), Consulting Biologist, *U. S. National Park Service, Box 378, Hilliard Road, Dumbarton, Va.*
- ALEXANDER, (EDWARD) GORDON, A.B. (Central), A.M., Ph.D. (Princeton), Associate Professor of Biology, *University of Colorado, Boulder, Colo.*
- ALLEE, WARDER CLYDE, S.B. (Earlham), S.M., Ph.D. (Chicago), Professor of Zoölogy, *University of Chicago, Chicago, Ill.*
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- ALLEN, EDGAR, A.M., Ph.D., Sc.D. (Brown), Professor of Anatomy, Yale University, *333 Cedar St., New Haven, Conn.*
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- ANDERSON, BERTIL G., A.B. (Augustana), M.S., Ph.D. (Iowa State), Instructor in Biology, *Biological Laboratory, Western Reserve University, Cleveland, Ohio.*
- †*ANDREWS, ETHAN ALLEN, Ph.B. (Yale), Ph.D. (Johns Hopkins), Professor of Zoology, Emeritus, *Johns Hopkins University, Baltimore, Md.*
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- AREY, LESLIE BRAINERD, Ph.D. (Harvard), Sc.D. (Colby), Robert L. Rea Professor of Anatomy and Chairman of the Division of Anatomy, *Northwestern University Medical School, 303 E. Chicago Ave., Chicago, Ill.*
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- BAITSELL, GEORGE ALFRED, B.S. (Central College, Iowa), M.A., Ph.D. (Yale), Professor of Biology, *Yale University, Osborn Zoölogical Laboratory, Yale Station, New Haven, Conn.*
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- CALVERT, PHILIP POWELL, Ph.D. (Pennsylvania), Professor of Zoölogy, *University of Pennsylvania, Zoölogical Laboratory, Philadelphia, Pa.*
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- CAUSEY, DAVID, A.B. (James Millikin), A.M. (Illinois), Ph.D. (California), Professor of Zoology, *University of Arkansas, Fayetteville, Ark.*
- CHALKLEY, HAROLD WILLIAM, B.S. (Mississippi A. and M.), M.A., Ph.D. (Johns Hopkins), Physiologist, Hygienic Laboratory, *U. S. Public Health Service, 25th and E Sts., N.W., Washington, D. C.*
- CHAMBERS, ROBERT, JR., A.B. (Robert), M.A. (Queen's University, Can.), Ph.D. (Munich), Research Professor of Biology, *New York University, Washington Square College, Washington Square, New York, N. Y.*
- CHAPMAN, ROYAL NORTON, B.A., M.A. (Minnesota), Ph.D. (Cornell), Director of the Experimental Station of the Pineapple Producers Cooperative Association and Dean of the Graduate School of Tropical Agriculture, *University of Hawaii, Honolulu, T. H.*
- CHARIPPER, HARRY ADOLPH, B.S., M.S., Ph.D. (New York), Chairman, Department of Biology, Associate Professor of Biology, *Washington Square College, 100 Washington Square East, New York, N. Y.*

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TOTALS

Life members,	5
Active members,	629
Associate members,	193
	—
Grand total,	827

NEW BOOKS AND PUBLICATIONS

THE FOUNDATIONS OF NUTRITION, by Mary Schwartz Rose, Ph.D., Professor of Nutrition, Teachers College, Columbia University. Third edition. Size, $6 \times 8\frac{1}{4}$ inches. Pages xi + 625. 116 illustrations in addition to numerous tables. Appendix. Index. Cloth. Price, \$3.50. The Macmillan Company.

"This book is written for those who wish to live more intelligently. An effort has been made to present within a small space some of the fundamental principles of human nutrition in terms which call for no highly specialized training in those natural sciences upon which the science of nutrition rests. The selection of topics and the relative amount of space devoted to each are based on much experience in presenting the subject of nutrition to beginners whose object is to be well informed as to the significance of food in daily life so that they may order their own lives more successfully and may have a better understanding of the part which nutrition plays in health in the world at large." The Publishers.

GENETICS AND THE ORIGIN OF SPECIES, by Theodosius Dobzhansky, Professor of Genetics, California Institute of Technology. 1937. Number XI of the Columbia Biological Series. Size, $6\frac{1}{2} \times 9\frac{1}{4}$ inches. Pages xvi + 364. 22 illustrations. Index. Bibliography. Cloth. Price, \$3.60. Columbia University Press, New York.

From the Preface: The present book is devoted to a discussion of the mechanisms of species formation in terms of the known facts and theories of genetics. Some writers have contended that evolution involves more than species formation, that macro- and micro-evolutionary changes may be distinguished. This may or may not be true; such a duality of the evolutionary process is by no means established. In any case, a geneticist has no choice but to confine himself to the micro-evolutionary phenomena that lie within reach of his method, and to see how much of evolution in general can be adequately understood on this basis.

INTRODUCTION AND GUIDE TO THE STUDY OF HISTOLOGY, for Students in Medical Schools and Colleges, by Avery E. Lambert, Ph.D., Professor of Histology in the School of Medicine, State University of Iowa. 1938. Size, $6\frac{1}{2} \times 9\frac{1}{2}$ inches. Pages xi + 542. 185 illustrations. References for suggested readings. Index. Fabrikoid. Price, \$5.00. P. Blakiston's Son and Co., Inc., Philadelphia.

"This 'Introduction and Guide to the Study of Histology' is the expansion of a former successful volume entitled 'A Guide to the Study of Histology and Microscopic Anatomy.' As its title implies, the present work is intended to be an introduction and not a text which undertakes, ambitiously, to include every aspect of the subject. Its purpose is to provide for the student who is beginning the study of medicine, and for those who wish to add a general survey of the subject to a more or less extended course in biology, the material and method of a 'working knowledge' of histology." From the Preface.

NEW BOOKS AND PUBLICATIONS

GENITAL ABNORMALITIES, HERMAPHRODITISM AND RELATED ADRENAL DISEASES, by Hugh Hampton Young, M.A., M.D., Sc.D., F.R.C.S.I., D.S.M., Professor of Urology, The Johns Hopkins University, Visiting Urologist, Brady Urological Institute, The Johns Hopkins Hospital. 1937. Size, 6×9 inches. 680 pages. 534 illustrations. Index. List of references. Buckram. Price, \$10.00. The Williams and Wilkins Company, Baltimore.

"The first comprehensive treatise in any language on the difficult but fascinating subject of related genital abnormalities, together with the amazing sexual disturbances that accompany them. It is, *inter alia*, a complete treatise on hermaphroditism, including hermaphroditism of all types, true and false, and the story of hermaphroditism in art. It is a medical document of first importance. Here one finds description of a wide range of sexual abnormalities, of new methods of surgical intervention, plastic repair, clinical treatment, all described in detail. Illustrated with 534 original drawings by William P. Didusch. But it is a biological document, and a human document as well. It explores the mysteries of the border-line between the sexes. It discloses extraordinary personalities. A book for every doctor—not merely to read but to ponder; a book for everyone scientifically interested in human life." The Publishers.

SHORT MANUAL OF REGIONAL ANATOMY, Written for the Medical Student as an Aid to a Rapid Revision of the Whole Subject, by J. A. Keen, M.B. (Lond.), F.R.C.S. (Eng.). Formerly Prosector and Demonstrator of Anatomy at King's College, London. 1937. Size, $5\frac{1}{2} \times 8\frac{3}{4}$ inches. Pages viii + 167. 76 illustrations. Index. Cloth. Price, \$2.00. Longmans, Green and Company, New York.

This manual is designed to minimize the effort necessary to the study of anatomy. Anatomical relations are shown by simple diagrammatic figures rather than by pages of description. All matters of general interest have been assembled in the first chapter on general anatomy which the student is urged to reread frequently. Omissions of descriptions of microscopical structure and function of organs make possible a great reduction in the size of the volume.

HANDBUCH DER BIOLOGISCHEN ARBEITSMETHODEN. Lieferung 469 Geh. Med.-Rat Prof. Dr. Emil Abderhalden. Abt. VII, Methoden der vergleichenden morphologischen Forschung, Teil 2, Heft 3. Spezielle Methoden anthropologischer Messung, by Theodor Mollison, München. 1938. Size, 7×10 inches. 160 pages. 183 illustrations. Bibliography. Price, RM 9. Urban & Schwarzenberg, Berlin and Vienna.

HANDBUCH DER ANATOMIE DES KINDES. Bearbeitet von Joseph Becker, Bremen; Otto Dragendorff, Greifswald; Ludwig Gräper, Jena; W. Gravinghoff, Münster i. W.; Albert Hasselwander, Erlangen; Friedr. Heiderich, Münster i. W.; Wilhelm Lange, Leipzig; Karl Peter, Greifswald; Wilhelm Pfuhl, Frankfurt A. M.; Richard Seefelder, Innsbruck; Sture A. Siwe, Lund; Georg Wetzel, Greifswald. Herausgegeben von Dr. Karl Peter, Professor in Greifswald, Dr. Georg Wetzel, Professor in Greifswald, Dr. Friedrich Heiderich, Professor in Münster i. W. Zweiter Band 5. (Schluss-) Lieferung. Charakteristik der wichtigsten Entwicklungsstadien des Kindes, von Professor Dr. G. Wetzel und Professor Dr. K. Peter, Greifswald. 1938. Size, 7×10 inches. 92 pages. Index. Price, RM 12.60. J. F. Bergmann, München.

SUPPLEMENT

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FIFTY-FOURTH ANNUAL SESSION

University of Pittsburgh, Pittsburgh. Pa.

April 14 to 16, 1938

ABSTRACTS

PAPERS TO BE PRESENTED

(The numbers in parentheses indicate the order in which the papers appear on the program of the meeting; the numbers in italics give the serial number of the abstract as printed herewith in alphabetical order of the authors' names.)

- (105) 1. *The behavior of living blood vessels of the rabbit during the reaction of anaphylaxis.* Richard G. Abell and Harry P. Schenck, Department of Anatomy, University of Pennsylvania. (Introduced by Eliot R. Clark.)

Direct microscopic observations in the moat chamber show that in rabbits sensitized to horse serum intravenous injection of this antigen or its introduction by way of the moat may be followed by arteriolar contraction, increased adherence of leukocytes to the blood vascular endothelium and leukocytic emigration. No contraction of the capillaries or venules occurred and no specific precipitates were observed in the blood vessels.

- (20) 2. *The vascular relations of the aortic-arch body in the adult dog.* William H. F. Addison and J. H. Comroe, Jr., University of Pennsylvania.

The aortic-arch body (glomus aorticum) lies between the aortic arch and the pulmonary artery in the adult dog. In our series of experimental animals, fifteen large adult dogs, a small artery was seen to leave the aortic arch at the level of origin of the brachio-cephalic artery: in fourteen animals on the dorso-caudal side of the aortic arch and in one near the orifice of the brachio-cephalic artery. When the tissue in the region of origin of the small artery was sectioned and examined microscopically an aortic-arch body was always found to be connected with a thin-walled branch of this small artery. The thin-walled vessel has but little muscle in its wall, in contradistinction to the parent artery, and soon after its origin gives off a series of branches which form the vascular part of the glomus aorticum. Serial sections of eight specimens have been studied. The presence of additional masses of similar tissue (as observed by Nonidez, '37, in neonatal dogs) is not excluded, but that the structure in the position described forms an important part of this neuro-vascular complex is shown by experiments in progress. These indicate that the aortic-arch body is a chemo-receptive organ which, in response to anoxemia, reflexly raises blood pressure and increases respiration.

- (129) 3. *An experimental analysis of the developmental properties of the somites of Amblystoma punctatum.* Howard B. Adelmann, Department of Histology and Embryology, Cornell University, Ithaca.

In *Amblystoma*, somite 2 forms the geniohyoid and first segment of the thoracicohyoid muscle; somites 3 and 4, the second and third segments, respectively, of the thoracicohyoid. A progressively slenderer geniohyoid and somewhat slenderer first two thoracicohyoid segments were formed when somites 2 to 4 were replaced (host and donor stage 25) by somites 3 to 5, and 4 to 6. The geniohyoid was absent, and first two segments of thoracicohyoid slenderer, after replacement by somites 5 to 7 (Adelmann and Maclean, '35).

When somites 2 to 4 (stage 25) are replaced by 5 to 7 (stage 30), ventral processes of grafted somites often grow down slightly in advance of normal 2 to 4; a geniohyoid forms from somites, but is slenderer than normal. When somites 5 to 7 are replaced by 2 to 4 (host and donor stage 25), grafted somites form segments 4 to 6 of thoracicohyoid.

In somites in question there is, therefore, apparently no mosaic of muscle-forming anlagen. In earlier experiments failure of geniohyoid to form from somite 5 was not due to inherent inability to form that muscle. The results may be interpreted as due to operation of 1) a time factor, since ventral processes of more caudal somites are formed progressively later, 2) a quantitative factor, 3) environmental influences, and possibly, 4) qualitative differences expressed as variable ability to adapt to new situations.

- (119) 4. *Functional organization of the medial geniculated bodies of the cat.*
H. W. Ades, E. A. Culler and Fred A. Mettler, University of Illinois and University of Georgia School of Medicine.

Localized bilateral lesions approximately 1 mm. in diameter were made in the medial geniculate bodies by means of the Horsley-Clarke instrument. Limens of auditory acuity were obtained for the seven frequencies representing the octaves from 125 to 8000 cycles by the conditioned response technique. The brains were prepared by the method of Marchi.

Hearing tests showed that localized lesions produce differential losses in acuity for different test frequencies. In nearly all cases some impairment was noted at all frequencies, but was maximal at only one or two. Lesions of different areas of the geniculates affected different frequencies maximally. Loci for the several frequencies were established as follows: 8000 cycles, dorsal quadrant; 4000, anterior; 2000, lateral; 1000, posterior; 5000, medial. It may be seen that the pattern of frequencies approximates a spiral with higher frequencies dorsally situated and circling ventrally to the lower ones. The organization of the geniculates thus apparently agrees with the pattern, so far as it is known, in other parts of the auditory pathway.

Study of Marchi sections of the brains reveals three principal types of connections of efferent fibers from the medial geniculates. The largest group consists of projection fibers passing to the sylvian cortex. A second type consists of recurrent connections to the lower parts of the pathway. Diffuse connections to other thalamic centers are also indicated.

- (69) 5. *Effects of estrogens in monkeys.* Edgar Allen, R. V. Worthington and N. W. Wawro, Department of Anatomy, Yale University.

Anterior pituitary stimulation of ovaries induces late follicular growth. Does ovarian reaction on pituitary play role in establishing menstrual rhythm? Ovariectomized immature monkeys require 500 R.U. estrogen in 10 days for menstruation. Subthreshold doses induce some 'sexual skin' reaction but no hemorrhage follows. Repetition of subthreshold dose is followed by menses. This 'carry over' effect of estrogen may be important in establishing the menstrual rhythm.

Tests of crystalline theelin by oral and parenteral routes indicates that the former requires ten times latter dose to induce an endometrial action which will be followed by menstruation.

The colchicine technique of accumulating mitoses in growing tissues clarifies the part played by cellular division in the response of genital tissues to estrogens. Ovariectomized monkeys treated with theelin for varying lengths of time and sacrificed $8\frac{1}{2}$ hours following injection of colchicine, showed varying growth rates according to 1) the stage of estrogenic stimulation, 2) the tissue elements considered, and 3) the region from which the tissue was taken.

Is morphology of uterine vessels a function of endometrial growth? Daily injections of theelin in mature ovariectomized monkeys induced progressive growth and edema of endometrium vascularized by capillary-like network of straight vessels. Following withdrawal of hormone, menstruating endometrium revealed compact cellular stroma and endometrium one tenth thickness of that after last injection of hormone and numerous coiled arterioles. Coiling seems to accommodate vessels to reduced endometrium confirming findings of Markee, Bartelmez and Daron.

- (182) 6. *The Golgi apparatus in the nerve cells of the mouse from youth to senility.* Warren Andrew, Department of Anatomy, School of Medicine, University of Georgia.

The Golgi apparatus in the nerve cells of the brain of the mouse has been studied in animals at various ages ranging from youth to senility in order to add to the cytological picture obtained by the Nissl technique of changes in these cells during the life history of the animal. Only pedigreed black mice have been used. The material was treated by the Da Fano silver technique, the brains of animals of different ages having been carried through the technical processes in the same container.

Definite differences in the architecture of the Golgi apparatus when the cells of young, middle-aged and senile animals are compared, have been found. These differences are constant or nearly so for all of the cells of any one group. Especial attention has been given to the large Purkinje cells. The cells of the cerebral cortex and of other regions have also been studied. The differences between nerve cells in different parts of the brain of young and old animals are very similar.

Nissl preparations made from the same brains from which the Da Fano preparations were made confirm the earlier findings on the differences in the Nissl substance of mice at different ages.

The changes in the Golgi apparatus when young and old mice are compared are more marked than those in the Nissl substance.

- (153) 7. *The history of the first somite in human embryos.* L. B. Arey, Department of Anatomy, Northwestern University.

The rise and fall of the first somite has been studied in a perfectly graded series of sixty human embryos, forty-five of which lie between 1 and 20 somites.

The first somite pair begins to show inferiority as early as the 5-somite stage. From 5 to 9 somites this inferiority is expressed by retarded differentiation only. During the 10- to 20-somite period, actual regression in size, shape and structure causes the first somite to decline and disappear.

The least altered of these small and atypical somites show definite histological inferiority, even though myotome, myocoele and sclerotome are fairly well represented. Following this, the external shape deviates from normal and regional differentiation largely fails, although there is still some trace of a myotome plate. Next, the somite is reduced to a spheroidal, unsomite-like mass which in section appears as a radiating rosette; lobation or subdivision may accompany this change. Finally, continued reduction produces a mere cell cluster, without definite organization. This preterminal stage has been observed as early as 13 somites. Differentiation is the mechanism by which somites wane and disappear.

The highest somite pair of most embryos with more than 20 somites is the original second pair, although sometimes the first somite resists decline longer than usual. Neuromeric relations aid in somite identification in these older stages. Recognition of the early loss of the first somite clears the way for future studies on the occipital contribution to the skull.

- (26) 8. *Premaxillary relationships in Old and New World monkeys.* M. F. Ashley-Montagu, Departments of Anatomy of the College of Dentistry and the Graduate School, New York University. (Introduced by Gustave J. Noback.)

In mature Old World monkeys the ascending processes of the premaxilla appear to be so prolonged that they frequently establish contact with the frontal between the nasal bone and frontal process of the maxilla. In New World monkeys the ascending processes of the premaxilla terminate at the infero-lateral angles of the nasal bones, occasionally passing below the inferior borders of the latter to terminate medially at the internasal suture. It is shown that the relations of the ascending processes of the premaxilla in Old and New World monkeys is actually the same, and that the apparent difference is not a real one, being due to the presence of a constant and hitherto unrecognized element which is present in the immature Old World monkey, and which in the mature animal undergoes union with the ascending process of the premaxilla, superior to which it is normally situated, and thus gives the appearance of being the continued frontal portion of the process. This element has been provisionally named the naso maxillary bone.

- (96) 9. *Time relationships in the growth and water exchange of the uterus following estrogenic stimulation.* E. B. Astwood, Biological Laboratories, Harvard University. (Introduced by F. L. Hisaw.)

Following a single subcutaneous injection of 0.1 gamma estradiol in oil into 21- to 23-day-old rats, changes in the weight of the uterus and its water content were followed at frequent intervals up to 96 hours. During the first 6 hours the uterus undergoes a 60% weight increase due chiefly to an imbibition of water. About one-half of this water is lost during the next 6-hour interval and there is, correspondingly, a decrease in uterine weight.

Following this there occurs a second increase in weight which, unaccompanied by a change in percentage water, reaches a maximum near the thirtieth hour. Later the uterine weight decreases and gradually returns to normal. This decline is preceded by a loss of water and during regression the percentage water falls to below the level of normal untreated uteri. The prompt edema of the uterus is interpreted as a direct action of estrogen on specific cells. The secondary rise, accompanied by mitotic activity and representing true increase in protoplasm is considered to be a secondary effect resulting from the estrogen-induced hydration. Detailed study of the early (6 hours) edema effect shows that between 0.0062 and 0.1 gamma estradiol, the weight increase bears a direct logarithmic relationship to dose. This close and constant agreement has permitted the development of a rapid and accurate assay for estrogenic substances.

- (9) '10.' *The role of the capsule in suprarenal regeneration.* Dan D. Baker and Ralph N. Baillif, Department of Anatomy, Louisiana State University Medical Center.

In a series of albino rats the right suprarenals were removed and the left enucleated, leaving the capsule and few glomerulosa cells intact. Colchicine was administered $9\frac{1}{2}$ hours before removal of regenerating left adrenals which were serially sectioned for study after postoperative intervals of 1 to 57 days.

After 24 hours, the capsule is found to be thickened and its vascularity increased. Mitoses appear in the capsule and cortical remnant; greatest numbers are encountered on the third and fourth days.

In regenerating glands there are unmistakable signs of migration of cells from the capsule and of their transformation into cortex. This is preceded by migration of capsular fibroblasts into the central blood clot. After the seventh day, the capsule becomes thin and assumes a normal appearance.

Cortical zones regenerate in the following order: 1) fasciculata, 2) reticularis, and 3) glomerulosa. Cell cords appear on the second day. As cortex forms the central clot is gradually replaced by fibrous tissue and large blood sinuses.

After the twenty-first day islets of pale staining cells which are morphologically identical with those of normal medulla are occasionally encountered in the intercordal connective tissue and along the line of operative incision. In almost every case nerve bundles can be traced to these regions. These cells do not give a typical chromaffin reaction. (See demonstration no. D2.)

(124) 11. *The hypoglossal nucleus.* John W. Barnard, Department of Anatomy, University of Michigan.

The hypoglossal nuclei of amphibians, reptiles, birds and mammals are analyzed, so far as possible, into natural subgroups. The varying degrees of development of the suprahyoid, hypobranchial musculature are interpreted in terms of these cell groups, with an attempt to relate the innervation of specific muscles to each group. The common pattern of the mammalian hypoglossal nucleus is deduced from the study of many species, all orders of mammals being represented with the exception of the cetaceans. A medial portion with three subgroups and a dorsolateral column with two subdivisions can be seen in the hypoglossal gray of practically all mammals. Evidence indicates that the cells of the medial nucleus innervate most of the intrinsic tongue musculature and the geniohyoid muscle, while the neurons of the dorsolateral column send their neuraxes to the major retractors of the tongue, i.e., the hyoglossal, styloglossal, palatoglossal and superior longitudinal muscles. The differences in pattern within the hypoglossal nucleus, as found in the mammals studied, are related, so far as possible, to variations in the development and function of the several tongue muscles. Experimental evidence of a localization pattern within the hypoglossal nucleus of the dog is brought forward and analyzed with respect to the normal subgroupings. The fiber connections of the hypoglossal nucleus of various forms are described and their significance discussed.

(42) 12. *Experimental changes in end feet.* Ruth I. Barnard, Department of Anatomy, The University of Chicago. (Introduced by Normand L. Hoerr.)

End feet were studied in the spinal cord of the cat by means of the Cajal reduced silver method and Regaud fixation, followed by staining with Mallory's connective tissue stain and aniline acid fuchsin methyl green. Two methods of approach were used in an effort to produce changes in the end feet of the spinal cord of the cat. In the first set of experiments end feet were separated from their cells of origin by a) motor cortex ablation, and b) hemisection of the spinal

cord. In neither of these sets of experiments could changes in the end feet on any of the cells of the spinal cord below the level of the lesion be detected among the widely varying, so-called normal end feet. In the second series of animals ventral roots were sectioned and the end feet studied on the chromatolyzed cells of the anterior horn after varying time. Progressive degenerative changes were seen in the end feet of the anterior horn cells which showed chromatolysis but upon no other cells. In this series, as well as the other series, and in normal animals, wide variations in end feet were seen, in size, shape, density of staining and frequency.

- (2) 13. *Plasmosin. An important constituent of protoplasm.* R. R. Bensley, Department of Anatomy, The University of Chicago.

By extraction of frozen-dried material first with 0.85% salt solution and, second, with 10% salt solution, it is found that the latter produces a clear extract which precipitates on dilution, on acidification, or on dialysis. The precipitate is in the form of a fibrous mass which under the microscope resembles, except for the absence of cells, a fresh mount of subcutaneous connective tissue. The precipitation is contingent on various factors, among which are the hydrogen-ion concentration, the concentration of salts, and the quality of salts. The function of this substance in connection with the reversible gelation of protoplasm are discussed.

- (188) 14. *Solubility studies of the secretion granules of the guinea pig pancreas.* S. H. Bensley, Department of Anatomy, The University of Chicago.

Pancreases from animals whose pancreatic ducts had been ligated for 3 weeks were dehydrated in vacuo at -40°C . Pieces of this tissue were treated with twelve organic and nine aqueous solvents and then embedded, sectioned and stained. The action of various aqueous solvents on fresh pancreas was observed directly under the oil immersion lens of the microscope. Fresh pancreas was extracted for 24 hours at refrigeration temperature 1) with distilled water and 2) with 0.85% salt solution. Pieces of each were then fixed in formol-Zenker, embedded, sectioned and stained.

The solubilities of the various secretion granules are discussed in respect to fixation and to extracts of the pancreas.

- (12) 15. *Effects of ultra-high radio frequency emanations upon the histological structure of the adrenal cortex of the albino rat.* Joseph G. Bernstein, Departments of Anatomy of the College of Dentistry and the Graduate School, New York University. (Introduced by M. M. Hoskins.)

With the cooperation of the Research Laboratories of the General Electric Company, a study was undertaken to determine the possible effects of ultra-high radio frequency emanations upon the endocrines, especially the adrenal cortex, and the probable role of the cortical lipid in body heat regulation.

A series of sixteen white rats, between 50 and 60 days of age, was treated for ten successive times with previously determined sublethal doses. Others were killed by lethal doses.

The endocrine glands of all animals and controls were removed at autopsy and preserved for study. The report to be presented concerns the conditions in the adrenal cortex.

Most of the material was sectioned at 10μ with the freezing microtome and stained with Sudan III. The rest was prepared by routine paraffin methods and stained with Harris' hematoxylin and triosin.

Cyanosis of the extremities, general hyperemia and gain in weight were the more noticeable gross effects. There was no apparent temperature tolerance built up by successive doses.

Sudan III stained adrenals showed the following consistent peculiarities: 1) A marked accumulation of lipid globules in the reticular zone. This zone is comparatively lipid-free in the normal condition. 2) An apparent hyperplasia of the cells in the fascicular zone, as indicated by the increased distance of the cell membrane from the nucleus.

The induction of sublethal internal tissue heat over long intervals of time results in an increased storage phase or in an increased secretion phase of the adrenal cortex. This is indicated by the more or less homeogeneous distribution of lipid over the entire cortex.

- (151) 16. *The effects of late fertilization upon the pre- and post-implantation stages of guinea pig ova.* Richard J. Blandau, William C. Young, Arnold Biological Laboratory, Brown University, and Departments of Primate Biology and Anatomy, Yale University School of Medicine.

Normal development of the guinea pig egg depends upon fertilization shortly after it is shed from the ovary. In 462 females inseminated approximately 8 to 26 hours after ovulation it was found that with the increment of age there is an increase in the number of sterile inseminations, a marked decrease in the litter size, and a great increase in the number of grossly pathological embryos which are aborted early in pregnancy. The abnormalities, which involve retarded development, fusion of blastomeres and cytolysis, are attributable to the age of the egg at the time of fertilization rather than a delay in implantation. Certain of these pathological ova become implanted. Photographs of unimplanted and implanted abnormal ova, and for comparison photographs of normal ova from the time of fertilization until the fourteenth day will be shown.

- (13) 17. *Alterations of the adrenal cortex in Amphibia bearing an excess or no pituitary tissue.* Raymond F. Blount, Department of Anatomy, University of Minnesota.

The close association of the adrenal cortex with pituitary disorders has been observed frequently particularly in connection with Cushing's syndrome of 'pituitary basophilism,' and Simmonds' disease. In the present experiments animals with additional pituitary tissue resulting from embryonic implanted anlagen and hypophysectomized individuals have been studied. Those individuals with additional pituitary tissue show a greater than normal amount of cortical tissue with cells which have an increase in cytoplasm. The hypophysectomized individuals show inverse changes. These cortical differences are correlated with changes already reported which are indicative of hypertensive state, such as

vasoconstriction, cardiac hypertrophy and a thickened basement membrane of the kidney glomerulus. The hypophysectomized individuals show vasodilation and a thin basement membrane. There is no question here but that the change in the cortical tissue is secondary to a primary pituitary alteration.

(185) *18. Mitotic rhythm in the albino rat: the kidney.* C. M. Blumenfeld, Department of Anatomy, University of Utah.

As the initial step in a study of the factors regulating cell division the occurrence of a mitotic rhythm is being investigated. For this purpose 240 normal male albino rats of the Wistar strain were killed at 28 days of age. Autopsies were arranged so that twenty rats were killed every 2 hours of a 24-hour period. Portions of the following structures were fixed immediately in Bouin's fluid: skin, tongue, submandibular gland, small intestine, liver, pancreas, brain, thymus, thyroid, adrenal, testis and kidney. Thus far the cortex of the kidney has been studied. The number of mitoses per thousand fields was construed as an index of the mitotic activity of a specimen. Values obtained to date indicate a fairly steady rate of activity from 2 A.M. to 6 P.M., but from 6 P.M. on there was a marked drop in the number of mitoses observed. At 10 P.M. the average number of mitoses per thousand fields was approximately one-half that of most of the other 2-hour intervals. By 12 midnight the number had again increased.

(60) *19. The size of cells in the motor area.* Gerhardt von Bonin, Department of Anatomy, University of Illinois.

The nuclear volume of the ordinary ganglion cells and of the giant pyramidal cells in the motor area of the brain of man, cebus and cat were measured in samples ranging from 100 to somewhat over 300 observations. The distribution of the ordinary cells are significantly skew and leptokurtic. The giant pyramidal cells are distributed normally. Throughout, variability is greater in man than either in the cat or in both cat and cebus. When compared with the ordinary cells the size of the giant pyramidal cells is relatively larger in man than in cebus, and in cebus than in the cat. The more complex the brain, the greater the variability of the size of its cortical cells seems to be.

(154) *20. A volumetric analysis of a 12-somite human embryo.* Edward A. Boyden, Institute of Anatomy, University of Minnesota.

This 23-day, 2.2-mm. embryo, obtained at hysterectomy by Dr. J. C. Litzenberg, is unique in that the entire chorionic vesicle and adjacent decidua has been sectioned serially at 8μ . By means of the paper weight method, the volume of the chorionic vesicle and associated intervillous space has been computed to be 295 mm^3 . Approximately one-fourteenth of this is chorionic cavity. The remainder is about equally divided between intervillous space and chorion. Fifty-eight per cent of the latter is trophoblast (80 mm^3), the preponderance of epithelium over mesoderm being due to the marginal proliferation of 'cell columns.' The volume of the embryo without its membranes is $\frac{1}{3}\text{ cu.mm.}$ (0.2), the umbilical cord 0.045 ,

the wall of the amnion almost as bulky, 0.03 mm³. The ratio of embryo, cord and amnion (0.277) to the combined chorion and intervillous space is 1:985. The pericardial cavity and its contents (0.043) is approximately one-fifth the volume of the embryo—almost equal to the umbilical cord. Myocardium and endocardium constitute one-sixteenth of the embryo. (The adult heart weighs only one-two hundredth of the body.) The brain is one-tenth of the embryo—in the adult it weighs only one-fiftieth—and three times the volume of the spinal cord. The right side of the embryo with respect to such constituents as the C. N. S., epidermis, placodes, pharynx and somites, is larger than the left side.

- (106) 21. *The effect, upon the red cells of the newborn rat, of administering anti-anemic substances to the mother during pregnancy.* Estelle Briesse and G. M. Higgins, The Mayo Foundation, University of Minnesota.

It has recently been shown (Stasney and Higgins, '37) that human or hog gastric juice administered daily to pregnant rats during gestation induced a reduction in the size and volume of the red cells of the rats born to these injected mothers. This report covers the results of a study in which a stomach preparation, ventriculin, was given orally and a liver extract (Eli Lilly) was given intramuscularly to pregnant rats, during a part or all of the gestation period. The blood of the young was examined at birth or as soon thereafter as possible. The total number of red cells per cubic millimeter, the percentage of packed red cells, and the size and volume of the red cells were determined. These data were contrasted with data obtained from newborn rats born of uninjected mothers fed a standard ration.

Both ventriculin and liver extract accelerated the maturation of the red cells in the young. The total number of cells per cubic millimeter was greater than in controls and the greatest diameter and volume of the red cells were less. Changes induced by liver extract were of less magnitude than those induced by either gastric juice or ventriculin. It is suggested that the normal physiologic macrocytosis of the newborn is due to lack of adequate anti-anemic principle during fetal development; and that the fetus derives its anti-anemic principle from the mother.

- (167) 22. *Pericellular nerve fiber terminations in the pars nervosa and pars distalis of the rat's pituitary.* Chandler McC. Brooks and I. Gersh, Department of Physiology and Department of Anatomy, The Johns Hopkins University.

The terminations in the pars nervosa of the nerve fibers which comprise the hypothalamico-hypophyseal system have not been clearly demonstrated. There is very little anatomical evidence that these nerve fibers end upon specific cells of the posterior lobe.

A careful search for nerve endings in the rat's pituitary yielded the following results. In fresh pituitaries of six 3-week-old rats perfused with methylene blue nerve endings were found. The numerous branches of large nerve fibers could be seen to terminate in a fine network enclosing the parenchymatous cells of the neurohypophysis. Pyridine silver preparations also showed these fine pericellular nerve terminations. With this method nerve endings were seen in four out of a total of thirty preparations, though the nerve fibers were well impregnated in almost all the cases.

The phenomenon of pseudopregnancy suggests that the pars distalis of the rat is subject to nervous stimulation. In the pyridine silver preparations terminations were found around both eosinophil and basophil cells. These endings enclosed a great part of the surface of the cells in a close-meshed network.

In six animals from which the superior cervical ganglia had been ablated, the nerve fiber pattern in the pars nervosa remained unaltered. Some nerve fibers were also present in the anterior lobe.

- (165) 23. *The effects of crystalline sex hormones on the differentiation of sex in Amblystoma punctatum.* R. K. Burns, Jr., Department of Anatomy, University of Rochester, School of Medicine and Dentistry.

Repeated injections of small doses of the crystalline hormones estrone and testosterone propionate (Schering) cause progressive reversal of sex in gonads of both sex types culminating in complete transformation. Thoroughgoing conversion of testis to ovary takes place more completely and more frequently under the influences of estrone than does the converse when testosterone propionate is acting. The histological stages are like those seen in reversal following parabiosis or grafting techniques.

On the contrary, the differentiating ducts and cloacal glands respond only to the action of the male hormone. Testosterone propionate produces great hypertrophy of the wolffian ducts and enormous growth of the cloacal glands in larval specimens; while comparable dosages of estrone have no visible effect on mullerian ducts even beyond metamorphosis, indicating either a marked difference of threshold for the two ducts during this period of development, or a difference in capacity to utilize the particular chemical forms of the hormones.

Control groups preserved at an early stage of the experiment and again at its termination, showed normal sex ratios and typical histological differentiation.

- (134) 24. *Studies on histogenesis in the regenerating limb of Amblystoma larvae.* E. G. Butler and W. O. Puckett, Laboratory of Comparative Anatomy, Princeton University.

Regeneration of a new limb in *Amblystoma* larvae depends primarily on, 1) an extensive series of dedifferentiative changes which the formed structures of the limb stump undergo, 2) the establishment of a regeneration blastema, which is a composite structure made up of a group of dedifferentiated cells, and 3) the growth and differentiation of the blastema to form the components of a new limb. Evidence has been found that an important physiological interaction takes place among the cells and groups of cells concerned with the three primary phases of regeneration. In particular, a delicate balance exists between cellular dedifferentiation and differentiation. Suppression of the establishment of an active blastema by x-rays, or ultra violet radiations not only prevents the development of new structures, but also permits a continued dedifferentiation of formed structures in the limb stump. The blastema is to be regarded, therefore, as a structure which arises by cellular dedifferentiation, later it is responsible for the cessation of dedifferentiation and finally it becomes the primary site for differentiation of new structures.

- (92) 25. *Some effects in animals of a plant estrogen separated from peas.* Hazel C. Cameron, West Virginia University. (Introduced by G. S. Dodds.)

Estrogenic action for mice and rats has been shown for the substance isolated by Leonian and Lilly from dried peas, and reported by them as markedly stimulating to sexuality in certain fungi, notably oomycetes. The distilled liquid, of deep yellow amber color and penetrating odor, is soluble in oils, and gives the deep red color by the modified Kober test of Cohen and Marrian for estrogenic substances. In preliminary trials on spayed mice, 0.3 to 0.6 gm. ($\frac{1}{4}$ cc.) of the material, proved fatal; later trials with half this quantity, showed positive oestrus 24 hours after the first injection, lasting for 4 days. In spayed rats, the effect was less striking, 1 rat unit being contained in approximately 0.5 gm. of material.

In a few immature rats tested, the substance produced no apparent hastening of sexual maturity. When given subcutaneously over a period of 10 days, it caused a decrease in the weight of testes, of 25 to 75%, and an increase in the weight of the spleen averaging 38% in males, and 220% in females. There was a slight and variable increase in the weight of the liver, and in the weight of the ovaries.

The material shows only a trace of vitamin A activity, and no substituting effect in animals for vitamin E. When tested upon capons, it showed no male hormone effect. It has been termed 'auxiphyle' by the authors, and since its effect is specific for only certain species of fungi, the possibility of using them as a test organism for estrogenic substances is considered.

- (104) 26. *An x-ray study of the blood island and vascular system of the Amblystoma embryo.* John A. Cameron and Katharine O. Mills, Department of Zoology, University of Missouri. (Introduced by Mary J. Guthrie.)

Amblystoma embryos were treated with 1000 r. in the neurula stage (Harrison stage 24). After 1 week at 22°C. the controls were found to have reached stage 37, with well-developed vascular systems including a functional heart with complete endothelium and many circulating blood cells throughout the body. The ventral blood island had all been converted into free cells although the ventro-posterior vessels still lacked endothelium. Individual blood cells were mostly ovals with compact, oval nuclei and with numerous but lightly staining yolk plates.

The x-rayed individuals had the ventral blood island wholly or partly differentiated as free cells but circulation had not been established and no circulating cells were found in the heart and anterodorsal vessels. These were, nevertheless, well formed with complete endothelial linings. The blood cells of the treated animals were still of early embryonic type, circular plates with multilobular, granular nuclei and many deeply staining yolk plates.

None of the twenty-two x-rayed embryos examined (series fixed daily for 7 days after exposure) gave evidence of connection between the retarded blood island and the developing heart. Thus we find that in the x-rayed embryos the heart and blood vessels with their endothelium are capable of differentiation without the aid of cells from the ventral blood island. This agrees with the conclusions of Stockard, Federici and Goss.

- (23) 27. *An ontogenetic study of the muscles of the opossum hand.* Berry Campbell, Department of Anatomy, University of Oklahoma School of Medicine.

This is a preliminary report of an attempt to evaluate the two theories of the homologies of the dorsal interosseous muscles. The theory of Ruge-Schomburg-Gräfenberg-Bardeen explains these dorsal muscles as migratory short deep flexors. In elaborate studies they have verified this in the human fetus. In the same year that Ruge's paper appeared, Cunningham advanced the view that the muscles are derived from a dorsal anlage and supported this thesis with analyses of the hands of marsupials. Subsequently, Young, Ribbing, and McMurrich elaborated the theory. As stated by McMurrich, the dorsal parts of the interossei represent the reptilian intermetapodial muscles.

In the opossum the two headed dorsal abductor muscles called dorsal interossei are seen to appear in the 25-mm. young as double structures inserting on both neighboring digits and thus consisting of both adductor and abductor elements. This effectively undoes any homology with the reptilian intermetapodial muscles—they probably represent the flexores digitorum minimi of amphibia. Such a remarkable atavism might be correlated with the unparalleled precocity of the hand in the marsupial embryo. The muscles are absent in the foot. This leads to the conclusion that the dorsal interossei of man are represented in the marsupials by certain of the short deep flexors and that through migration one anlage gives rise to all interossei as claimed by Ruge.

- (39) 28. *Experimental histology of nerve fibers.* Eben J. Carey, Department of Anatomy, Marquette University School of Medicine.

The nervous system of the cat, rabbit, rat and frog is subjected to different chemical and mechanical depressants and excitants. The purpose is to detect whether or not there are histologic differences in the relatively normal, hyperactive and inactive nerve fibers. The cauda equina and sciatic nerves are fixed and stained while intact in the animal, with formaldehyde-thionine 2 to 7 days and subsequently put in dioxane before embedding in paraffin. The relatively inactive nerve has a variable diminution in the number and size of the axonal varicosities and also in the number of fibers that possess these swellings. When 1 to 2 gm. of magnesium sulphate per kilogram of body weight are injected subcutaneously there is profound muscular palsy corresponding to a rapid diminution in the size of the nerve fibers. There is a rapid reduction of metabolism related to the rate of dehydration. The nerve fibers stimulated to hyperactivity by mechanical, chemical, electrical and thermal stimuli have a greatly increased number and size of the axonal dilatations and also in the number of fibers that possess the alternate enlargements and constrictions. These axonal bulgings are assumed to be produced by mechanical explosion pressure waves in a microcapillary filament of watery protoplasm related to rhythmic changes of chemical action of metabolism. (See demonstrations nos. D5 and D6.)

- (19) 29. *Accessory parathyroids in a colony of albino rats.* Simon B. Chandler, Department of Anatomy, West Virginia University School of Medicine.

In 1925 Hoskins and Chandler reported results which indicated that accessory parathyroids occur rather infrequently in the albino rat. In order to further test the frequency of accessory tissue in the albino rat colony at Loyola University School of Medicine, check the reliability of the operative procedure, and to correlate the experimental history with the anatomical findings the neck regions of sixty-eight animals were serially sectioned. Parathyroidectomy was done at 100 days and the animals lived from 10 to 14 months after operation. Some bore several litters and the others were subjected to fifteen fasting periods at intervals of 1 week.

Of the sixty-eight animals, fifteen were normal controls. Accessory parathyroid tissue was found in one control and four experimental animals. In two, the accessory tissue was associated with the thyroid. These were not examples of incomplete extirpation, but small masses of tissue somewhat distant from that removed by operation. In one animal a small parathyroid was embedded in the strap muscles of the neck and in the other it was cephalad to the thyroid.

These results confirm earlier findings—accessory parathyroids do not vitiate results in a large and well-controlled series. They further indicate the necessity for histological examination of all tissue removed at the time of operation. Even when this precaution is taken a small error is probable.

- (51) 30. *Sympathetic nerve fibers in the alveolar nerves and nerves of the dental pulp.* Kermit Christensen, Department of Microanatomy, St. Louis University School of Medicine.

Dissections of the cat's neck and head show that only small rami of the external carotid plexus join the branches of the maxillary and mandibular nerves which provide the innervation of the dental pulps. The majority of the smaller nerve fibers in sections of the alveolar nerves appear to be of the myelinated type. A smaller number of these fibers are unmyelinated and these are considered to be sympathetic. After unilateral intracranial section of the maxillary or mandibular nerves, some nerve fibers, most unmyelinated, remain in the respective alveolar nerves while a few fibers of similar type or none are left in the dental pulps of the same side. Removal of dental pulps from the teeth of one side causes chromatolysis of a slightly greater number of superior cervical ganglion cells on the operated side than on the unoperated side.

- (40) 31. *Microscopic observations on new growth and medullation of peripheral nerves in the living mammal.* Eliot R. Clark and Eleanor Linton Clark, Department of Anatomy, University of Pennsylvania.

The growth and behavior of peripheral nerves in transparent chambers installed in rabbits' ears have been studied microscopically with high magnifications in the living animal, with particular emphasis upon medullation. Among the many observations are the following.

There is a marked variation in the extent of new formation of nerves, depending upon mechanical conditions, nearness of cut end to region of new growth, and frequency with which the region is subjected to conditions which reproduce a 'growth promoting' environment.

Visible medullation begins at the site of the Schwann cell and progresses in both directions from it.

Isolated stretches of medullation may start at several Schwann cells simultaneously.

Nodes of Ranvier are usually absent at first but may develop later.

Myelin degeneration occurs very rapidly over the entire stretch of nerve beyond a mild pressure injury; the Schwann cells may persist, and remedullation may occur, at times centripetally.

Apparently nerves grow out when the growing ends are favored by a growth promoting environment, and not because there is an area of tissue which 'needs' them. They rarely supply a new area as richly as do blood capillaries and connective tissue cells, and they may be lacking altogether. This is attributed to their slow start caused by the degeneration which occurs proximal to the cut.

- (125) *32. Effects from electrical stimulation of the cerebellum of cats.* Sam L. Clark, Department of Anatomy, Vanderbilt University School of Medicine.

With sterile precautions permanent electrodes were planted in contact with the cerebellum in cats which were allowed to recover. While the results of stimulation could be varied by alterations in the stimulating current, particular regions responded to stimulus with a constant pattern. The response from most areas stimulated could be observed to occur in three phases: the first, brief in duration and coincident with the stimulus; the second, also brief, ordinarily following immediately on cessation of the stimulus; and the third, prolonged in character, and involving consecutively different regions of the animal's musculature. When stimulation of an area produced motion of the head the movement of the second phase appeared as a rebound, opposite in direction to that of the first; that is, if the head turned to the right with the stimulus, it turned to the left when the stimulus ceased. The motions of the third phase (continuing 5 to 10 minutes) were relatively slow in character, and consisted usually in a tonic lifting of the homolateral then contralateral forelimb, accompanied or followed by turning of the head to the homolateral, then contralateral side; after this, lifting of the homolateral, then contralateral hind limb, accompanied or followed by hooking of the tail to the homolateral then contralateral side. When the electrode was in the mid-line both forelimbs, and later both hind limbs were affected simultaneously.

- (17) *33. A study of the adrenal-parathyroid relationship.* F. W. Clausen and C. M. Blumenfeld, Department of Anatomy, University of Utah.

The 10-week-old male Wistar albino rats used in this investigation were divided into groups of four. In each group one animal was adrenalectomized and received no further treatment; one, adrenalectomized and given an option of 2% sodium chloride solution or ordinary tap water to drink; the third, adrenalectomized and injected daily with eschatin; the fourth, the control, was subjected to a dummy operation. When death appeared imminent in the untreated, adrenalectomized rat of a group, the group was autopsied. This was done, on the average, 7 days after operation.

At the time of autopsy each rat was anesthetized, blood was obtained from the heart, and the serum calcium content was determined by the Clark-Collip modification of the Tisdall method. The volume of the parathyroid glands was measured from serial sections by the paper outline method.

In comparison with the serum calcium value for the controls, the untreated tests had an 8.20% lower value; the salt-treated tests, an 8.96% lower value; and the eschatin-treated animals, a 0.16% higher value.

The average volume of the parathyroid glands of the untreated tests was 4.17% less than that of the controls, but, statistically, the difference was probably not significant. Studies of the parathyroid volume of the other groups are in progress.

- (34) 34. *The cranio-facial hafting zone and the maxillary tuber in mammals and man.* W. Montague Cobb, Anatomical Laboratories, Howard University and Western Reserve University.

The contrasting growth patterns of cerebral and visceral crania necessitate complex developmental adjustments where face is anchored to braincase. Three subdivisions of this hafting zone are described: a median hafting, the most important, comprising the union of nasal, premaxilla, maxilla, lacrimal and palatine with frontal, and the union of palatine with orbito- and ali-sphenoids; behind and sometimes confluent with this, a posterior hafting, the pterygopalatine union; and a lateral hafting, the temporomalar union. The lateral (sloth) or posterior (musk deer) may be absent or vestigial. Braincase attains adult size early and is the stabler cranial unit during most of postnatal growth. Hafting zone adjustments with the slower growing face are related principally to strengthening of the fixation of maxilla as the successional dentition develops and to expansion of the paranasal sinuses. Growing permanent teeth distend the maxilla as a fetus the womb. The maxillary tuber is a posterior protrusion which lodges the developing permanent molars. As the latter erupt the tuber recedes variably. According to requirements of dental specialization the tuber may: project backward unsupported into the infratemporal fossa (four-horned antelope, camel); be pulled laterally and sacrificed for strong malar arches (tiger); or obtain support medially from palatine and posterior hafting by apposition to palatine (wart hog, tapir, man). In elephant the tuber has to be supplemented by palatine for dental accommodation.

- (29) 35. *The effect of pregnancy and lactation on growth in the rat.* H. H. Cole and G. H. Hart, Division of Animal Husbandry, University of California.

In studies on superfecundity in immature rats (Am. J. Physiol., vol. 119, p. 704) Cole observed that rats following parturition were slightly heavier than non-bred litter-mate controls. In extending this observation one group of ten rats were allowed to go through a series of pregnancies and lactations, a second group of ten through a series of pregnancies without intervening lactations and, finally, the third group of ten were maintained as non-bred litter-mate controls. Following the fifth to ninth parturition at 10 to 12 months of age the first group were approximately 30% and the second group 24% heavier than non-bred litter-mate controls (395, 375, and 304 gm. respectively). Both pregnant groups were also distinctly larger as determined by linear measurements.

This faster rate of growth during pregnancy is related to an increased appetite manifested as early as 48 hours following copulation, long before there is any appreciable demand for nutrients by the embryos. Food consumption during pregnancy is about 30% greater than in the controls and is relatively constant throughout gestation. During mid and late lactation rats consume about three times as much as controls. During the first 14 days of lactation there is a fairly rapid weight increase.

Results from another group, heretofore unmentioned, show that the accelerating effect of pregnancy on growth is diminished after the three hundredth day.

- (90) 36. *Some quantitative studies on experimentally induced oestrus in the spayed guinea pig.* Vincent J. Collins, John L. Boling and William C. Young, Arnold Biological Laboratory, Brown University, and Departments of Primate Biology and Anatomy, Yale University School of Medicine.

Sexual receptivity was produced in spayed guinea pigs by subcutaneous injections of estrin and progesterone as described below. The interval between estrin and progesterone administration is the conditioning period, that between progesterone and the beginning of heat is the latent period. 1) 50 I.U. estrin followed by 0.2 I.U. progesterone with conditioning periods 12 to 108 hours. Heat occurrence decreased from maximum of 80% at 24 hours to 7% at 108 hours; 2) 200 I.U. estrin followed by 0.2 I.U. progesterone with conditioning periods 24 to 120 hours. Heat occurrence decreased from maximum of 87% at 24 hours to 14% at 120 hours; 3) 50 I.U. dihydroxyestrin-benzoate followed by 0.2 I.U. progesterone with conditioning periods 1 to 8 days. Heat occurrence decreased from 100% at second and third days to 17% on eighth day.

Supplementary observations were as follows: a) Despite quantitative and qualitative differences in estrin dosage, neither latent period nor length of heat was materially different. b) Latent period and length of heat show an inverse correlation. c) Latent period and length of heat remained fairly constant even when percentage of animals in heat decreased greatly.

In (4) and (5) the conditioning period was kept constant, but progesterone and estrin dosage was varied respectively. The percentage of animals in heat plotted against hormone variations give sigmoid curves.

- (128) 37. *On the origin of the neural crest.* J. LeRoy Conel, Department of Anatomy, Boston University School of Medicine and Massachusetts Memorial Hospitals.

Embryos of *Bdellostoma*, *Squalus*, *Torpedo* and chick present evidence that the neural crest arises as a hollow evagination of the dorsal part of the alar plate in a manner similar to the evagination of the optic vesicle. The optic vesicle and the neural crest are, therefore, homologous. In early stages the cavity of the evaginated crest is continuous with the cavity of the neural tube. The evagination of the alar plate proceeds in a cephalo-caudal direction. The optic vesicle appears first; next to appear is the trigeminal crest, while the medullary folds are still separated; then closely follows the evagination of the balance of the cephalic part of the crest. The spinal portion of the crest evaginates after the neural tube is closed and appears first at the rostral end.

An actual or potential cavity is present in all parts of the developing crest and its processes and in the early stages of the ganglia.

- (52) 38. *Vascularity in the brain and in the hypophysis of the leopard frog.*
E. Horne Craigie, Department of Biology, University of Toronto.

The average diameter of the capillaries in ten regions of the brain of the frog is $5.6\ \mu$ (not corrected for shrinkage), which is similar to that in the dogfish but greater than in the rat. The vessels in the corpus striatum neuropil and the molecular layer of the cerebellum are narrow.

The most vascular part studied in the brain is the cochlear nucleus. The vestibulo-cerebellar centers and the forebrain are relatively poor.

The poorest regions found were the medial longitudinal bundle and the neuropil of the primordium piriforme and the corpus striatum. Thus the hypothesis that regions rich in neuropil are highly vascular is not confirmed.

The frog is intermediate in cerebral vascularity between the dogfish and the newborn rat.

In the hypophysis the average diameter of the vessels is least in the pars anterior ($7.8\ \mu$), greater in pars intermedia ($9.4\ \mu$) and pars nervosa ($9.0\ \mu$). The condition in the latter thus differs markedly from that in mammals. In length of capillaries, the pars nervosa is richest, followed in order by pars anterior, pars tuberalis, and pars intermedia.

The pars anterior is more vascular in males.

Areas of capillary walls are greater in pars nervosa and in pars anterior than in brain tissue, and the areas in pars nervosa and pars intermedia are greater than in the cat. (See demonstration no. D9.)

- (86) 39. *Effect of progesterone on cell division in the circular muscle of the rabbit's uterus.* William Crandall, Department of Anatomy, University of Rochester, School of Medicine and Dentistry. (Introduced by George W. Corner.)

A comparative study has been made of the circular muscle of the rabbit's uterus at 24-hour intervals during the first week after mating. The experiments include a series of normal pregnancies, a series of rabbits castrated 18 to 24 hours after mating, a series of rabbits castrated 18 to 24 hours after mating and subsequently treated with subcutaneous injections of various dosages of estrin or progesterone in sesame oil.

Circular muscle of normal pregnant rabbit uterus shows a peak of 117.4 mitoses per square centimeter 48 hours after mating, and then gradually subsides to zero mitoses 144 hours after mating. Untreated rabbits castrated 18 to 24 hours after mating show 22.3 to 71.5 mitoses per square centimeter of circular muscle 48 to 72 hours after mating and none thereafter. Estrin treated rabbits show a few mitoses only after a prolonged high dosage (500 I.U. a day for 4 days). Progesterone treated rabbits show a curve of mitosis rising from zero 24 hours after mating to as high as 119.8 to 216.5 mitoses per square centimeter in rabbits given 0.2 mg. per day for 2 days and killed 72 hours after mating, and gradually subsiding again to zero 120 hours after mating.

- (122) 40. *The accessory olfactory bulb in phylogeny.* Elizabeth Caroline Crosby and Tryphena Humphrey, Department of Anatomy, University of Michigan.

Where present, the accessory olfactory bulb of representative higher vertebrates, with its associated vomeronasal nerve, shows a relative constancy of pattern although exhibiting a wide variation in size even in closely related forms.

Herrick has described an accessory olfactory bulb in amphibians.

In available turtle brains the accessory bulb varies but in general is well developed. In Squamata it is either highly developed or absent. Thus in snakes (garter snake, water moccasin, copperhead) and in certain lizards (Varanus, Gila monster) the olfactory bulb consists of a huge, semilunar accessory olfactory formation projecting into the ventricle, with the remaining thin wall and rostral tip of the bulb occupied by a scanty olfactory formation. Certain other lizards (Anolis, horned toad) and the American alligator have no accessory bulbs.

The birds studied have no accessory bulbs.

The marsupial (opossum) has a well-developed accessory bulb. In ungulate material (McCotter) and in certain carnivore and rodent series the accessory bulb is very large. It is greatly reduced in insectivores (shrew), and lacking in Chiroptera (bat) and, apparently, in adult primates (macaque, man). The macaque material was provided by faculty research funds of the Horace H. Rackham School of Graduate Studies, University of Michigan.

The accessory olfactory bulb represents a way-station for vomeronasal impulses to postbulbar centers. Consequently its relative development becomes an important factor in forebrain phylogeny.

- (109) 41. *Poly-lobation as a result of work and time ageing of the polynuclear neutrophiles.* Arthur M. Crosman and Harry A. Charipper, Washington Square College, New York University.

Dead fetuses retained in utero offer opportunity to study the effect of phagocytizing and autolysing on neutrophile lobation. Rats were used in which one dead fetus had been retained from 12 hours to 8 days. Polynuclear counts were made in both uterine blood vessels and comparable regions in the uterine cavity. The normal colony polynuclear index was shown to be 1.15 (Yeager and Haterius, '30). This was constant in blood vessels of the uterus of pregnant animals. The polynuclear cells in the blood vessels of the uterus of experimental animals showed a tendency to right deflection after 24 hours retention. This was followed by a left swing after 48 hours, then by a shift to the right, and after 8 days retention the index was 1.24. In the uterine cavity, the index showed a steady right shift through 48-hour retention, after which there appeared to be a slight movement to the left, followed by a most apparent rise after 96 hours, while the 8-day period indexed 3.04. This shift indicated that during continued retention the leucocytes in the uterine cavity became more lobated. This is offered as confirmation that, in vivo, lobation is a factor of time and experience ageing (Ponder and Climenko, '34).

- (115) 42. *The pretectal region of Pseudemys scripta troostii*. Alice O. Curwen and Ruth N. Miller, Department of Anatomy, Women's Medical College of Pennsylvania. (Introduced by Hartwig Kühlenbeck.)

The pretectal region has been studied in *Pseudemys scripta troostii*, which is a reptile showing a relatively primitive, undifferentiated arrangement. The following cell masses could be identified: nucleus posterodorsalis; nucleus pretectalis, pars dorsomedialis and pars ventrolateralis; nucleus lentiformis mesencephali, pars magnocellularis and pars parvocellularis; area pretectalis of Papez; three nuclei of the posterior commissure, nucleus dorsalis, nucleus nucleus interstitialis with pars superior and pars inferior, and nucleus interstitialis tegmentalis. The paracentral gray is most prominent rostrally, where it blends with the nucleus posterodorsalis.

A wax plate reconstruction has been made to show the relative position of these nuclei.

Although the material was studied for cytoarchitecture, the fiber connections have been followed as far as the material permits.

- (27) 43. *A roentgenological study of the morphological age changes in the human laryngeal cartilages*. Maxwell Dauer, Departments of Anatomy, College of Dentistry and the Graduate School, New York University. (Introduced by Gustave J. Noback.)

A series of 100 larynges (ninety white males; ten white females) ranging in age from 29 to 90 years were radiographed in order to determine, more definitely, the beginning, extension and completion of the transition from cartilage to bone of the human laryngeal cartilages. The process of ossification of the thyroid cartilage, as found in this study, differs from that of the reports of previous investigations. In the male, from the initial centers in the inferior cornu, posterior border and the inferior thyroid tubercle, three independent radiations from each of these sites results in final ossification. In the female, these three extensions are not found. In three male specimens in which a chronic disease was the cause of death, the corniculate cartilages showed a definite stage of pre-ossification. A hitherto unreported center was observed on the dorso-medial aspect of the arcus of the cricoid cartilage.

In general, ossification increases with age. However, due to the wide range of variability of the ossification of the cartilages, no definite correlation between the extent of the process and the chronological age of the individual may be made. Since some chronic diseases seem to retard the normal process while others appear to accelerate it, the process of osseous change as manifested by the individual cartilages is probably an index of physiological rather than chronological age. (See demonstration no. D10.)

- (41) 44. *Ratios of myelinated to unmyelinated fibers in sciatics and nerves to gastrocnemius after 3 months' regeneration in M. rhesus.* H. A. Davenport, Herman Chor and D. A. Cleveland, Departments of Anatomy and Nervous and Mental Disease, Northwestern University, and Department of Surgery, Marquette University.

Estimates of the number of fibers shown by osmic acid and silver stains were made on the 3-month regenerated sciatic just below the level of the femoral trochanters and on the gastrocnemius-medial sural cutaneous fascicle in the popliteal space. Although a distance of 7 cm. separated the two locations, the ratio of myelinated to unmyelinated nerve fibers was similar. In seven animals the ratio varied from 1 myelinated to 1.2 unmyelinated to 1:3.3 (av. 1:2.0) in the sciatic while ratios varied from 1:1.1 to 1:6.3 (av. 1:2.1) in the gastrocnemius-cutaneous fascicle. These data are believed to indicate that fibers which are going to acquire a myelin sheath do so within a very short time after their regrowth into the degenerated stump, because a definite preponderance of unmyelinated fibers in the more distal location was not found. No close correlation between the extent of myelination in the proximal and distal locations of a given animal was seen, thereby showing that the regrowth of fibers was more of an erratic than orderly process. About 64% of the normal number of fibers (all types) was restored in the sciatic at the end of the 3-month period of regeneration.

- (174) 45. *The occurrence of a second type of acidophile cell in the anterior pituitary of the female cat and its relation to sexual activity.* Alden B. Dawson and Harry B. Friedgood, Biological Laboratories, Harvard University and the Department of Physiology, Harvard Medical School.

The anterior pituitary of the cat has been investigated using the method previously described in the study of the rabbit pituitary: formalin-corrosive sublimate fixation followed by the azan modification of Mallory's staining method (Friedgood and Dawson, '37). The ordinary acidophiles are stained with orange G, the special cells react with azocarmine. This special carmine cell was not found in any of the glands of immature kittens of both sexes, and was rarely found in adult males, but appeared more numerous in one chronic castrate examined. These cells were also absent in the pituitaries of anestrus females examined from October to January. They are, however, present during sexual activity. The present data on the occurrence of these cells during the first estrus, after mating and during recurrent estrous periods are still very incomplete. These cells are apparently not derived directly by the modification of ordinary acidophiles but seem to differentiate from chromophobes. (See demonstration no. D12.)

- (44) 46. *Structural variation in axon hillocks of motor and associational neurons in the cat.* Q. B. DeMarsh, Anatomical Laboratory, Northwestern University Medical School. (Introduced by W. F. Windle.)

Serial sections of spinal cord and cerebral cortex of cats killed by perfusion with Orth's fluid were stained with toluidin blue. Individual cells were studied in their entirety by means of photographic maps.

Of 196 large spinal and cortical motor neurons, 140 showed axon hillocks characterized by a varying degree of fragmentation and dissolution of the chromatic substance; few lacked it entirely; some hillocks were very small and others so large that they occupied more than half the perikaryon including one or more dendrons. In thirty-seven cells, axons could be identified by their even contour and deficiency of chromatic substance but they arose from cones whose Nissl pattern differed in no way from that of the perikaryon. In thirteen cells, neither axon nor hillock could be found.

Of 238 associational type neurons of the spinal cord, only two had characteristic axon hillocks. In sixty-one, the axons could be identified but cones were filled with chromatic material. The remaining 175 cells had no characteristic hillocks and all processes were so similar in structure that axons could not be identified.

- (82) 47. *Spider (Ateles geoffroyi) and howler (Alouatta palliata) monkey ovaries at various stages in the reproductive cycle.* Edward W. Dempsey, Department of Anatomy, Harvard Medical School. (Introduced by George B. Wislocki.)

Twenty-six female spider and five female howler monkeys were shot in the field and their reproductive tracts immediately fixed. The condition of the ovaries shown in serial sections was correlated with the phase of the reproductive cycle at death. Specimens were classified as oestrous when copulation was observed before death or when sperm were found in the vaginae. Lactation was inferred when females were carrying young. Pregnancy was determined by dissection of the uteri.

The ovaries of both forms contain many epithelial masses whose cells, during the follicular phase, are indistinguishable from the granulosa cells of atretic follicles. At the time of ovulation luteinization of these cells occurs, resulting in almost solidly luteinized ovaries. Luteinization of these elements occurs more rapidly than does luteinization in the granulosa of the ruptured follicle itself. Ovulation and luteinization apparently occur before the end of the oestrous period, since of eight specimens killed shortly after copulation seven had ovulated.

During lactation many medium-sized follicles, but no lutein tissue, were found. Conversely, during pregnancy there was an almost complete suppression of follicles, while more extensive luteinization occurred even than during normal cycles.

The excessive luteinization suggests that large amounts of luteinizing hormone are produced during the lutein phase and pregnancy. This assumption is strengthened by the observation of atresia and maturation spindles in follicles and ova during these periods.

- (36) 48. *Tubulation in normal and rachitic bones.* G. S. Dodds and H. C. Cameron, West Virginia University.

The characteristic form of a long bone (a tubular shaft terminating in enlarged extremities) is maintained during growth, as is commonly recognized, by the continual removal of superficial bone from the curved portions below the epiphyseal cartilages. This process is known as tubulation. This local removal is accomplished by numerous osteoclasts in the periosteum overlying the ends of the trabeculae of this region.

During rickets, as seen in the head of the tibia of rats with experimental rickets, these trabeculae early become covered with a thick layer of sub-periosteal osteoid and the osteoclasts disappear. Tubulation is thus prevented. During the further progress of rickets, the bone undergoes some elongation in the region of the epiphyseal cartilage, elongation which, in the absence of tubulation, causes the enlarged extremity to become somewhat cylindrical, instead of having its normal taper toward the shaft. The extremities of such bones are probably no thicker than normal, but the enlarged portion is longer.

During healing the sub-periosteal osteoid is removed, the osteoclasts reappear and the tubulation process is resumed. Thus the superfluous bone is soon removed and the normal form of the extremity is restored by about the time that the normal internal structure is attained.

- (43) 49. *Aberrant ganglion cells as a source of intact fibers in the central ends of severed dorsal roots.* Donald Duncan and E. S. Crocker, Department of Anatomy, University of Texas.

Operations designed to show the presence or absence of 'recurrent' and efferent fibers in the lumbo-sacral roots of the cat were performed on thirty-five animals. Occasional intact fibers were found in the central ends of the severed dorsal roots that could not be accounted for by incomplete section, regeneration or anastomosis with adjoining roots. The chance finding of ganglion cells in a teased preparation of a degenerated root which contained a few normal fibers led to an investigation of these extra-ganglionic cells. Aberrant dorsal root ganglion cells were found to be rare in the cat, but fairly numerous in the dog. In the dog over one-half of the length of a lower lumbar dorsal root is extra-dural in contrast to the more usual mammalian arrangement in which nearly all of the root is intra-dural. Most of the aberrant cells in the dog were found in the extra-dural portion of the root but they are more numerous intra-durally than in the cat. Survival of these cells and their processes following extra-dural section in the dog has been demonstrated. Many of the surviving cells show definite chromatolysis. These scattered cells in the dorsal roots account for most of the normal fibers that were found in severed dorsal roots.

- (28) 50. *The human renal fascia in an unusually homogeneous group.* John N. Edson and Edgar D. Congdon, Department of Anatomy, Long Island College of Medicine.

Studies were made of individuals who were of the male sex, of the white race, of an age usually between 45 and 55, whose muscular and connective tissue structures had not wasted, and who were of a medium condition of adiposity, and who were of the sthenic constitution as far as could be told from external inspection. The structure of the renal fascia varied within a smaller range than described by most other workers. This is apparently due to our high degree of selection. It seems probable that although only fifteen bodies were studied, the norm for our anatomical group has been demonstrated. Complete series of $\frac{3}{8}$ inch frozen sections of all bodies were studied. Tracings of the connective tissue structures of each section were prepared by means of a special device.

The fascia forms a cone with an apex below the iliac crest. The anterior cranial free border in most instances terminates opposite the middle cranio-caudal third of the kidney and does not reach the diaphragm. It is usually completed on the posterior side by part of the psoas and quadratus lumborum fasciae. It does not cross over the vertebral column. It gives off wings to the paracolic gutter, and to various viscera.

Adaptations of structure to function are considered.

- (205) 51. *The pigments and color of living human skin.* Edward A. Edwards and S. Quinby Duntley, Department of Anatomy, Harvard Medical School, Boston, and Department of Physics, Massachusetts Institute of Technology, Cambridge.

The spectro-photometric measurement of the living human skin allows an analysis of its pigments and serves as a basis for an objective specification of its color. Such measurements, without the aid of the human eye, were made of normal young adults, including male and female members of the white race, and a limited number of males of the dark races. The factors responsible for normal skin color include five pigments: melanin; an allied diffuse material which we have named melanoid; oxy-hemoglobin; reduced hemoglobin; and carotene. The color produced by each of them varies, but they are all similar in reflecting more of the wave lengths near the red end of the spectrum. A blue component is added by the optical property of scattering, caused by the turbidity of the epithelium. The regional distribution of the pigments follows a quite regular pattern which will be described. The female skin shows less melanin and reduced and oxy-hemoglobin, but more carotene than the male skin. There is also a minor sex difference in the pattern of pigment distribution. The idea that the racial differences are due to variation in the quantity of melanin present is here affirmed.

- (14) 52. *Effect of splenectomy on the adrenal glands of the male albino rat.* Linden F. Edwards and Munroe W. Palestrant, Department of Anatomy, The Ohio State University.

Fifty male rats (263.8 gm. mean body weight) were splenectomized and were sacrificed 1 month later along with their litter mate controls (260 gm. mean body weight). That splenectomy produces an increase in the weight of the adrenal glands is evidenced by the fact that the mean weights of the left adrenal, right adrenal and both adrenals combined were 13%, 32% and 23% greater, respectively, than those of the controls. Moreover the mean of the relative percentage weight of the adrenals to the body weight was 11% greater in the test group than in the control.

A preliminary histological examination of adrenal glands from the test group indicates increased vascularity and lipid content and decreased chromaffin substance.

- (24) 53. *The function of leg muscles in walking.* Herbert Elftman, Department of Zoology, Columbia University. (Introduced by William K. Gregory.)

The work which the leg muscles do in walking can be determined by recording the force which the foot exerts against the ground by means of an especially designed apparatus, a motion picture of the individual being taken simultaneously. From these data the forces which the muscles exert on the trunk, shank, thigh and foot and the rate at which they do work can be found. It is possible to distinguish between the function of muscles in 1) contributing energy by contraction; 2) transmitting energy from one part of the body to another; and 3) receiving energy when they are stretched before contracting. A consideration of these factors throws some light on the difference in function between one-joint muscles and those which traverse more than one joint. See demonstration no. D17.)

- (164) 54. *Studies of possible sex reversals produced in the developing chick by x-rays.* J. M. Essenberg and Anton Zikmund, Department of Anatomy, Loyola University School of Medicine.

The material for this report is derived from four groups of experiments: 1) eggs irradiated prior to incubation, 2) eggs treated at 5 to 12 days of incubation, 3) young chicks irradiated during the first 3 weeks after hatching, 4) developing chicks x-rayed twice, during incubation and after hatching. The dosage varied from 40 to 600 r. The ovaries were preserved for study during the first 22 days after hatching.

Initial injuries to the ovarian follicles occur in all ovaries irradiated with 200 or more roentgen units. Major damages to the ovary is caused by double irradiation in which the sum of the two dosages is at least 300 r. The ovum of the young follicle is affected first. While the ovum atrophies, the follicular cells multiply and fill the follicle. The resulting spherical cell masses are the most common constituents of the ovarian cortex. Cords of varying shapes and sizes result from growth of the cell masses. The cell cords become tubules by the formation of a lumen.

The tubules so formed, have a marked resemblance to the seminiferous tubules of the young chick. The developmental history of the two structures is similar. The formation of an extensive tunica albuginea adds a third male characteristic to the injured ovary.

- (120) 55. *The effects of combined red nucleus and pyramidal lesions in cats.* Byron Evans and W. R. Ingram, Department of Anatomy, State University of Iowa.

The symptoms following destruction of the major portions of the red nuclei or interruption of the rubrospinal tracts in cats include dysmetria and increased extensor tonus. In time these defects are largely overcome. Partial section of a pyramid in our experience leads to variable results: in some cases manifest increase in extensor tonus and deficiency in the postural reactions in the contralateral limbs, with occasional slight paresis; in others these effects were slight or absent except for deficiencies in postural reactions. There is rapid compensation.

One or both pyramids were sectioned at various time intervals following production of red nucleus lesions. If the effects of the latter had been well overcome, pyramid section caused return of the hypertonus, ataxia and dysmetria, with loss of postural reactions, in the contralateral limbs, with milder ipsilateral effects. These changes were in time partly overcome by compensation. Somewhat similar reemphasis of symptoms occurred when the red nuclei were damaged subsequent to pyramid section. Coincidence of the acute stages caused rather marked symptoms which diminished in intensity and which did not approach in degree those of decerebrate rigidity.

It is suggested that both the pyramidal and rubrospinal systems participate in the complex mechanism regulating muscle tonus and economy of movement in cats and that each may act to compensate to considerable extent for loss of the other. Injury to both, however, may be further compensated for by participation of still other systems.

- (171) 56. *Stimulation of deciduomata around threads on administration of lactogenic and adrenocorticotrophic hormones.* Herbert M. Evans, Miriam E. Simpson and Kaisa Turpeinen, University of California.

Anterior pituitary extracts potent in lactogenic or adrenocorticotrophic properties were invariable stimulants for the formation of deciduomata in adult normal or hypophysectomized rats (twenty-one normal, fourteen hypophysectomized; dose 60 to 240 systemic pigeon units of lactogenic in 10 days, 0.5 to 3 normal rat units of adrenocorticotrophic hormone). Uninjected normal or hypophysectomized controls did not show deciduomata on insertion of threads. The presence of the adrenals is not necessary for the reaction but the ovaries are essential. These pituitary extracts were devoid of gonadotropic effects as determined by all known methods at many times the doses necessary to produce deciduomata. The ovaries of the treated hypophysectomized rats did not show growth of follicles or change in deficient interstitial tissue but the cells of the corpora lutea were enlarged as compared with those of untreated rats. The production of deciduoma is interpreted as due to increased secretion of progesterone produced by the lactogenic and adrenocorticotrophic hormone.

- (37) 57. 1. *Human temporomandibular joint, areas of alisphenoid (epipterygoid) and pterotic (epitympanic) invasion.* 2. *Bony outgrowth on shaft of left femur in adductor magnus (occupational).* *Egyptian charioteer (?)*. Thomas H. Evans, Long Island Medical School and New York Medical School.

1. Study of types of temporomandibular joint, some with area for alisphenoid and for pterotic. This invasion occurs mediad and produces a further triangular area within the fissure of Glaser. Two hundred thirty-four crania studied and foetal stages dissected.

2. This femur (S-62) from Brooklyn Central Museum, attributed to the XVII-XIX dynasty, appears to show the results of pressure of the left knee, from youth, due to an occupation. No pathology. The indices will be contrasted with those of normal femora. Third trochanter present, showing activity of gluteus maximus against resistance. Nutrient canals of femur, first and third perforating. Platymeria. (See demonstration no. D20.)

- (186) 58. *Triploidy in the common newt, Triturus viridescens*. Gerhard Fankhauser, Department of Biology, Princeton University.

In various species of amphibians, triploid embryos and larvae have been found occasionally in normal, preserved material (G. Hertwig, Bataillon, Daleq, Fankhauser, Fankhauser and Kaylor). The repeated occurrence of such cases indicated that it might be possible to discover triploid individuals among living larvae and to follow their development.

In young larvae of the common newt, *Triturus viridescens*, the tail tips were amputated, fixed and stained in toto with haemalum. Accurate chromosome counts could be made in mitotic figures in the epidermis of the tail fin. So far, the chromosomal condition of 100 larvae has been determined. Ninety-six were found to be diploid, with twenty-two chromosomes; the remaining four, all from the same mother, showed thirty-three chromosomes in several mitoses. In these triploid tail tips the nuclei in various tissues appear to be larger than normal.

In the living larvae, the triploid condition was indicated by the larger size of the melanophores. The general body size did not show a consistent increase over the normal. One triploid only was, from an early larval stage on, much larger than the controls. Three of the four animals are completing metamorphosis at the present time (March 3rd).

These four cases offer valuable material for the study of the effects of triploidy on development and particularly on sex, which have not yet been investigated in vertebrates. (See demonstration no. D32.)

- (61) 59. *On a developmental problem presented by the brain of a mentally defective child*. Patrick Alexis Fitz-Gerald, Department of Anatomy, University College, Dublin, Ireland. (Introduced by George L. Streeter.)

Description of the cerebral hemispheres of an eighth month male child, which are almost smooth in the central and parietal regions, and but poorly fissured in the lower temporal regions. The other cortical areas are approximately normal. A report will be given on the areas of cortical lissencephaly, correlating the arrest of cortical histogenesis with the stunting of sulcus formation.

- (55) 60. *Biochemical changes associated with onset of secretion in the fetal chorioid plexus. Evidence of a secretory mechanism.*¹ Louis B. Flexner and Robert D. Stiehler, Departments of Anatomy and Ophthalmology, Johns Hopkins University.

Distribution ratios between blood plasma and cerebrospinal fluid of fetal pigs indicate that the fluid is ultrafiltered until the forty-fifth day of gestation; later it is secreted. At this transition period the following changes appear in the chorioid plexus: 1) Before secretion, oxidation-reduction potentials of stroma and epithelium are identical. With secretion, stroma becomes approximately 100 millivolts more negative than epithelium. Concentration of indophenoloxidase appears equal in stroma and epithelium of presecretory stage: in secretory stage, oxidase is limited to epithelium and is increased in concentration. 2) Before

¹ This investigation was supported in part by a grant from the John and Mary Markle Foundation.

secretion acid and basic dyes diffuse from stroma to epithelium or vice versa and are unaffected by asphyxia. With secretion basic dyes pass from stroma to epithelium but not from epithelium to stroma readily. Acid dyes pass from epithelium to stroma but not readily in the opposite direction. With asphyxia, these dyes behave as in the presecretory stage. 3) The isoelectric point of the basement membrane between epithelium and stroma has been determined by following the effect of pH changes on the diffusion of acid and basic dyes.

These observations support the theory that in the secretory stage an electric current between stroma and epithelium causes cations to move from stroma to epithelium and anions in the reverse direction. The energy for this current is supplied by oxidative and reductive processes.

- (159) 61. *The effects of prolonged injections of testosterone in recently hatched alligators.* Thomas R. Forbes, Departments of Anatomy, The University of Rochester School of Medicine and Dentistry and Johns Hopkins School of Medicine. (Introduced by Lewis H. Weed.)

A total of 10 mg. of testosterone divided into seventy-two equal doses was administered by intraperitoneal injection over a 91-day period to each of fifteen alligators (fourteen females, one male). There were six female and three male control animals. Serial sections were made of the gonads and gonoducts. The oviducts of the injected females showed a moderate mucosal hyperplasia, the differentiation of a muscular stratum, and a striking gross increase in length and diameter. Such transformations as took place in the experimental ovaries were too variable to be considered significant. The clitorides were unaffected.

In the single experimental male there was a great hypertrophy of the penis and an advanced development of the vestigial mullerian ducts. The experimental testes were twice as large as those of the control animals. Wolffian ducts and mesonephroi were not affected in either sex.

- (113) 62. *The amygdaloid nuclei and fiber connections in the cat.* Clement A. Fox, Department of Anatomy, University of Michigan. (Introduced by Elizabeth C. Crosby.)

The amygdala subdivides into medial, lateral and anterior nuclear groups. Cortical, medial and central nuclei constitute the medial group. The cortical nucleus, circumscribed grossly by the circular amygdaloid fissure, intervenes between hippocampal and pyriform cortices. The larger medial nucleus lies lateral and then anterior to the optic tract. Dorsomedial to it, along the stria terminalis, is the central nucleus. The stria terminalis connects with all nuclei of this group.

Lateral and basal nuclei form the lateral group. The large lateral nucleus, intimately associated with the external capsule (from which it receives cortico-amygdaloid fibers), sends fascicles to the contralateral nucleus through the anterior commissure. Medial to the lateral nucleus is the basal nucleus, which posteromedially receives strial fibers and anterolaterally sends fascicles into the overlying longitudinal association bundle. This bundle includes fibers from lateral and basal nuclei and distributes chiefly to the preoptic area, but in small part joins the medial forebrain bundle.

The nucleus of the lateral olfactory tract, the intercalate mass and the anterior amygdaloid area constitute the anterior group. The spherical, antero-medially-lying nucleus of the lateral olfactory tract connects with its contralateral nucleus through the commissural component of the stria. The globular intercalate mass, situated rostral to the basal nucleus, contributes to the anterior commissure. The diffuse anterior amygdaloid area is associated with the preoptic region and the diagonal band.

- (76) 63. *The influence of estrogens upon the mammary glands of monkeys.* W. U. Gardner, and G. van Wagenen, Departments of Anatomy and Obstetrics and Gynecology, Yale University School of Medicine.

The mammary glands of male or immature ovariectomized monkeys developed completely when estrogens were injected (2000 to 8000 i.u. weekly). The period of treatment required for a morphologically complete development was from 6 to 7 months. Recent studies, using colchicine, have enabled the study of the details of growth activity during different stages of development. During the early stages the peripheral ducts of the breast grew very rapidly. Mitotic activity was accentuated in the peripheral ducts and in the surrounding stroma. Later, starting near the nipple the terminal parts of the smaller lateral branches established other centers of proliferation and lobules formed. In monkeys receiving large amounts of estrogen these two processes occurred simultaneously after an initial period of duct growth. The stages in the experimental development differed in some respects from those in normal females as determined by one of us (G.v.W.).

Mammary glands of monkeys receiving estrogen still showed mitotic activity (colchicine method) after 8 months of treatment. At this time the uteri and cervixes showed little evidence of proliferation. The uterine stroma had become quite dense and fibrous. The epithelium was not actively growing and the cells were vacuolated. The epithelium of the mucous glands of the cervix was almost completely replaced by a stratified squamous epithelium. The proliferative activity of the breast tissue was apparently more persistent than that of the uterus when estrogens were administered continuously for long times.

- (15) 64. *Pseudopregnancy in the adrenalectomized ferret.* Robert Gaunt and Harry W. Hays, Washington Square College, New York University and Princeton University.

The adrenalectomized ferret, like the dog, was found to live without any treatment during pseudopregnancy. Anestrus, adrenalectomized ferrets lived from 3 to 10 days, average 6 days. The left adrenal of the ferret is easily accessible surgically but the right is broadly attached to the posterior vena cava and sometimes is partly embedded in the liver. Complete adrenal removal, including the excision of macroscopic accessory tissue in two animals, was effected in eleven of twelve cases with an electro-cautery. Minimal maintenance doses of cortical extract were 0.7 to 1.0 cc./day on a milk and fresh meat diet with a 2% Na-salt supplement.

All adrenalectomized, cortical hormone-treated females came into estrus spontaneously between January and April. Early estrus was induced in some with prolans or pregnant mare serum. Estrus did not reduce but apparently increased the cortical hormone requirement. When, however, pseudopregnancy was induced either by sterile matings or gonadotropic agents, the animals lived in nine cases for 25 to 46 days, average 35 days, with no treatment at all. Such life extension failed to follow gonadotropic hormone injections in three cases, but was invariable after spontaneous estrus and mating.

Survival of the adrenalectomized ferret can be prolonged, as in the cat but not the dog, by the Squibb APE (growth) preparation—an effect probably independent of the gonads.

- (45) 65. *The innervation of the striated muscle of the swim bladder of the sea robin (Prionotus carolinus).* John B. Gaylor, the Marine Biological Laboratory, Woods Hole and the Johnson Foundation, University of Pennsylvania. (Introduced by G. de Rényi.)

The intramural muscle of the swim bladder of the sea robin is striated and subserves the function of noise production by throwing the tough wall of the organ into vibration. The nerve supply to this muscle is mainly derived from the first and second spinal segments and only to a small extent from the vagus. The preponderating motor innervation is through a system of medullated nerves which branch profusely, ultimately lose their medullated investment and end in small reticulate plaques on the muscle fibers. A second innervation is derived from a ganglionic system the cells of which lie irregularly distributed between the muscle fibers. Post-ganglionic fibers can be traced from their cells of origin to endings of the same morphological characteristics as those derived from the somatic system. It is suggested that individual muscle fibers are innervated from somatic and autonomic sources. The nerve endings of both systems are not in the form of organized sole plates.

- (11) 66. *Some problems in the maintenance of tissue cultures of endocrine organs to be used for transplantation purposes in cases of specific endocrine deficiency.* George O. Gey, Department of Surgery, Johns Hopkins Medical School.

In a series of preliminary studies already published in several papers by Doctors Stone, Owings and Gey attention has been called to the possibility of adapting by tissue culture methods specific types of cells to a tissue culture medium composed largely of the prospective recipients' plasma and serum. The work as a whole has progressed sufficiently far that we are now justified in reporting elsewhere that over one-third of a series of sixteen patients with marked parathyroid tetany are well from 2 to 6 years after homologous grafting with tissue cultures, and an equal number similarly treated have been definitely improved. In a series of animal experiments (dogs) a large number of successful homologous grafts of thyroid cultures have been demonstrated whereas only a few of the parathyroid grafts were successful.

In the present paper some of the technical aspects of the problem of maintaining pure strains of thyroid and parathyroid cells indefinitely in continuous culture are discussed. In the whole of the grafting work to date this problem seems to be, by far, the most important and should be extended. In a separate demonstration, motion pictures showing the cytologic appearance and cultural behavior of the actual cells used for transplantation purposes in a series of human and dog experiments are illustrated. (See demonstration no. D23.)

- (163) 67. *The development of thymus IV in the dog.* M. C. Godwin, Departments of Anatomy and Histology, Medical School of West Virginia.

The embryo of the dog develops four pharyngeal pouches and an ultimobranchial body. Early in development the fourth pouch is usually absorbed within the walls of the complex IV but this is not always true. It seems probable that the thymus IV develops from the material of the fourth pouch. The thymus IV is irregular in its occurrence. When present it tends to be present on both sides. The degree of development on opposite sides also tends to be similar. Reticulation and lymphocytic infiltration appear quite late in fetal life. Ultimobranchial remnants which have not been induced to form thyroid do not form thymus. Nothing was found to indicate that thymus arose from any other source than material which was derived from the fourth pouch.

- (111) 68. *The reticulo-endothelial system and hormone refractoriness. III. Experiments with pregnant mare serum.* Albert S. Gordon and Harry A. Charipper, Washington Square College, New York University.

The ovaries of normal 25- to 30-day-old female rats respond to single daily injections of 5 R.U. pregnant mare serum (Gonadin, Cutter Laboratories) by increasing in size, reaching a maximum weight ranging from 172 to 200 mg. in about 15 days. This is followed by regression, normal ovarian weights being attained within 2 to 3 months despite continued treatment. The ovaries of the injected splenectomized litter mates of these animals show an increased growth, apparent as early as 10 days subsequent to spleen removal, and reach a maximum size in about 25 days, at which time they ranged from 362 to 425 mg. against 92 to 140 mg. in similarly treated controls. At this time, the ovaries of the splenectomized animals are more heavily luteinized than those of the injected controls. With continued treatment, the ovaries of the splenectomized animals begin to regress rapidly after 30 days and also reach normal size within 2 to 3 months. This is due, presumably, to reticulo-endothelial compensation. It is possible to prevent this regression to some extent, by causing a partial 'blockage' of the remaining reticulo-endothelial elements with trypan blue injections. Experiments show that the sera of 19- and 26-day injected splenectomized animals possess a smaller quantity of the antagonistic principle for the injected hormone than the sera of similarly treated controls.

All animals used in these studies were free of *Bartonella muris*, a factor already shown to greatly influence reticulo-endothelial activity.

- (33) 69. *The anatomy and genetics of some skeletal variations in the rabbit.*
E. L. Green and P. B. Sawin, Arnold Biological Laboratory, Brown University.
(Introduced by William C. Young.)

Museum studies of homeotic variations of the axial skeleton in which one or more morphological units assume the function and appearance of their immediate neighbors have been interpreted by various authors on the basis of cranial or caudal transmutations having phylogenetic significance. The present data, consisting of five generations (1000 animals) from a cross between two selected strains of rabbits differing with respect to several morphological features of the axial skeleton, substantiate the belief that the cranial transmutations with certain exceptions are genetically dominant to the caudal and simulate a mendelian segregation, thus providing a tangible mechanism for such phylogenetic changes. In view of significant correlations between the degree of expression of an extra pair of ribs, an additional presacral vertebra, and also an extra ventral spinous process, it seems reasonable to assume that the same genetic forces operate over the entire region including both thoraco-lumbar and lumbo-sacral borders. That the expression of these forces is also affected by minor genetic and environmental influences is indicated by the response of the variations to selection, and by significant and consistent departures from expected mendelian proportions in parental stocks and subsequent generations. Since morphological and genetical units do not coincide, it may be concluded that the morphological units are merely the manifestation of more fundamental physiological forces which may have to do with growth and differentiation.

- (169) 70. *Structural and functional ovarian response of hypophysectomized adult rats treated with gonadotropic hormones.* Roy O. Greep, Biological Laboratories, Harvard University.

Adult female rats were treated with luteinizing preparations for 5 days prior to hypophysectomy. The ovaries lost 45% of their weight in the first 5 post-operative days; 52% by 15 days and 57% by 40 days. The initial drop was due to follicular involution; the corpora lutea persisted, however, and later comprised the great bulk of the ovaries along with a few primordial follicles. All experimental treatments were started on the fifteenth day after hypophysectomy. Pituitary FSH stimulated follicular growth, and maintained continuous vaginal estrus. The matured follicles underwent atresia without becoming luteinized and were replaced. The persisting lutein bodies were not affected. Pituitary LH produced no follicular growth and no estrus, but the corpora lutea were destroyed. With simultaneous FSH and LH therapy the growth of a first crop of follicles and the involution of the persisting corpora proceeded independently. These follicles became luteinized on the fourth to the sixth day following which another crop of follicles arose and the newly formed corpora regressed. After about the thirteenth day these cyclic processes became erratic and the gonads underwent profound regression due presumably to exhaustion of the follicular apparatus and destruction of the last-formed corpora lutea. The vaginal smears showed periods of estrus which coincided with periods of follicular dominance.

Injections of estrogen-free pregnancy urine extract produced long-continued estrus in the absence of any follicular growth. Damaging the persisting corpora lutea with LH previous to the urine injections prevented the appearance of estrus. The evidence indicated that persisting corpora lutea secrete estrin when stimulated with pregnancy urine.

The persisting corpora lutea proved to be non-functional and attempts to stimulate them to produce the progestational hormone have thus far been unsuccessful.

- (147) 71. *The dimensions of the pelvic inlet in 700 white women.* William Walter Greulich, Department of Anatomy and the Adolescence Study Unit, Yale University School of Medicine.

The dimensions and configuration of the pelvic inlet were determined by Thom's method of roentgen pelvimetry in 600 primigravid, white women in the Obstetrical Clinic of the New Haven Hospital. The anteroposterior, or true conjugate, diameter was found to exceed the maximum transverse diameter (dolichopellic type) in 15.2% of the cases. The maximum transverse diameter either equalled the anteroposterior or exceeded it by no more than 1 cm. (mesatipellic type) in 44.5% of the cases. The maximum transverse exceeded the conjugate by from 1.1 to 3 cm. (brachypellic type) in 35.8%, and by more than 3 cm. (platypellic type) in 4.4% of the cases. Among 100 students of the Yale University School of Nursing, who represented a somewhat different racial stock and a more privileged economic group than did the clinic women, 37.0% were dolichopellic, 46.0% were mesatipellic, 17.0% were brachypellic, and none were platypellic.

In modern textbooks of anatomy and of obstetrics, the normal female pelvis is described as having an inlet the maximum transverse diameter of which exceeds the anteroposterior by approximately 2 cm. Since this type of pelvis was found in less than one-third of the clinic women and in only 9% of the student nurses in our series, it appears that the prevailing concept of the shape of the inlet of the normal female pelvis is in need of some revision.

- (137) 72. *On the duration of the polarization process in the ear primordium of *Amblystoma punctatum*.* Edmund K. Hall, Department of Anatomy, University of Louisville School of Medicine.

Experiments were performed in which the ear ectoderm was transplanted from stages well before anteroposterior determination (from neural plate and fold embryos, stages 13 to 18) to stages immediately after such determination (stages 21 to 23) with reversal only of the anteroposterior axis of the graft (orthotopic heteropleural dorso-dorsal operations). In 37% of the ninety-seven cases, disharmonic (contralateral) labyrinths formed with the correct dorsoventral orientation and in 20%, reduplicated ears. These indicate that in a certain proportion of the cases, an incipient anteroposterior polarization exists in the primordium before determination of this polarization at fusion of the neural folds (stages 20 and 21). In 14%, harmonic (ipsilateral) ears formed and in 29%, epithelial vesicles without criteria for laterality. In another series (100 cases), ear ectoderm from stage 21 and 22 donors was transplanted to stage 27 to 37 hosts with

reversal only of the dorsoventral axis (orthotopic heteropleural dorsoventral operations). In 71%, labyrinths developed with criteria for the normal, harmonic (ipsolateral) condition; the remaining cases are not sufficiently differentiated to have criteria for laterality. These results indicate that no incipient dorsoventral polarization is present in the primordium before determination (at stages 23 to 25), but that dorsoventral polarizing stimuli from surrounding tissues continue their activity long after determination.

- (179) 73. *Induction by male hormone substance of a state in experimental animals resembling that of the adrenal syndrome in humans.* James B. Hamilton, Department of Anatomy, Yale University School of Medicine.

The phenomena characteristic of both the human female presenting an adrenal syndrome and female animals receiving large amounts of synthetic male hormone consist essentially of 1) masculinization or virilism, 2) elimination of cyclic function of the reproductive system and 3) if the state be induced in embryonic life, the production of pseudohermaphroditism in which the internal organs are feminine, the external masculine, and the vagina ends in the urethra below the bladder at the müllerian tubercle.

These similarities between the condition experimentally produced in animals and those naturally occurring in the human adrenal syndrome appear to be more than coincidental, for 1) minute details of each are alike, such as formation of not merely prostatic glands but of just certain portions of the male prostate, and the production of not only a penis-like clitoris but one with a hypospadiac-like condition and 2) the degree of affectation in each is parallel in different age groups, with progressively greater abnormality in the earlier ages of prenatal onset. Male hormone as an agent responsible for the pathogenesis of this condition receives support from studies of testicular grafts, from assays of adrenal tissue and from urine assays of patients with this syndrome. If the resemblance between the experimentally produced and the naturally occurring conditions be real, it would mean that the pathogenesis of much bodily change in cases of adrenal tumor or hyperplasia is brought about by male hormone substance and would afford a technique for experimental induction of the phenomena produced in the adrenal syndrome. (See demonstration no. D25.)

- (155) 74. *An anencephalic human embryo 10 mm. long.* A. E. Harbeson, Department of Anatomy, Queen's University. (Introduced by G. H. Ettinger.)

The embryo is estimated to be about 6.5 weeks old. Its crown-rump length is 10 mm. There is complete absence of the calvarium. Extending from the occipital area to the lumbar region is an angiomatous mass. Anterior to this mass is a lighter colored structure which appears to be brain tissue. This consists of three parts, the two halves of the cerebrum and the mid-brain. The cerebrum is flattened out and extends over the supra-orbital margins. The eyes are well developed. The nasal pits are shallow. The developing ears are not seen. The embryo's body is straight. The tail is absent. Only the external features are described since the specimen is to be saved for the museum. Anencephaly is believed to result from an angiomatous growth originating in the tela choroidea of the fourth ventricle.

- (1) 75. *Further investigation of the factors concerned in the development of the ear.* Ross G. Harrison, Yale University.

The three main factors in the development of the amphibian internal ear have been studied in a series of experiments which eliminate the possible effect of the reconstituted half of the hind-brain present in the cases previously reported (Anat. Rec., vol. 64, 1935). To the three factors there studied (ectoderm, mesoderm, myelencephalon) a fourth, the position of the rudiment, has been added. Transplantation experiments with early neurula stages of *Amblystoma*, involving each of these factors alone or each in any combination with others (sixteen combinations in all) have been made, from which the conclusion is drawn that all four, except possibly the mesoderm, are required to produce a normal labyrinth, but that certain combinations are more effective than others. The inductive action of mesoderm has not been proved effective in abnormal position. In normal position, however, after bilateral removal of the myelencephalon, small but unmistakable ear vesicles develop out of abdominal ectoderm used to cover the wound. If the presumptive auditory placode is also removed, only the induced ears develop, but if the placode is left in place, then two ears on each side, one from the graft and one from the host, are formed.

- (77) 76. *Pregnancy in the monkey continues after castration.* Carl G. Hartman, Department of Embryology, Carnegie Institution of Washington, Baltimore.

Two rhesus females castrated in 1937 on the ninetieth day of gestation carried their fetuses to full term.

Monkey, no. 457, was castrated on the forty-sixth day of gestation; no. 657 on the thirty-fifth day of gestation; no. 466 on the thirty-first day of gestation. All three pregnancies are now (March 4th) progressing normally, 78, 52 and 59 days, respectively, after castration.

As the corpus luteum of the rhesus monkey regresses sharply in the fourth week of gestation (Corner, Bartelmez and Hartman), the results stated above might theoretically be expected. They are also in consonance with clinical experience.

- (22) 77. *A comparison of the effects of thymectomy, thymo-ovariectomy and ovariectomy.* E. I. Hashimoto, Department of Anatomy, University of Utah Medical School. (Introduced by C. B. Freudenberger.)

Four groups, each containing twelve Wistar albino rats, were subjected to thymectomy, ovariectomy, thymo-ovariectomy and control operations at 25 days of age and autopsied at 90 days. Biometrical methods were used in determining quantitative differences in the four groups.

Thymectomy appeared to produce lighter organ and body weights, but the changes were not significant with the exception of the thyroid gland which was heavier. Histologically, there was no evidence that these thyroid glands underwent hyperplasia, but there was questionable evidence supporting the contention that the weight alteration might have been due to intra-thyroid thymic rests.

Ovariectomy produced significantly heavier weights of body, head, integument, femur, humerus, spinal cord, hypophysis, thyroid, thymus, stomach, intestines, submaxillary glands, kidneys, heart, lungs, liver, and spleen. Likewise, the length of body, tail, femur, and humerus were significantly longer than control animals.

Thymo-ovariectomized animals differed from ovariectomized ones only in the fact that the weights of spinal cord, kidneys, heart, lungs, liver, and spleen were not significantly altered.

Weights of brain, eyeballs, suprarenal glands, and alimentary group were not significantly changed as a result of any of the three operations.

A comparison of percentage differences showed that the general trend of the changes produced in the organs and body was as follows: thymectomy produced lighter organs; ovariectomy, markedly heavier organs; thymo-ovariectomy, heavier organs, but less so than ovariectomy alone.

- (57) 78. *Cerebral development of Amblystoma*. C. Judson Herrick, Department of Anatomy, The University of Chicago.

Over 250 specimens of *A. punctatum* and *A. tigrinum* from first motility to feeding stages (Harrison, 33 to 46) have been examined and seriated by Coghill's physiological and Harrison's morphological criteria, with comparison of the two species. The range of variability and of deviation from exact parallelism of the anatomical and physiological series was explored. The sequence of maturation of cerebral nerve fibers is recorded in correlation with physiological stages. Peripheral and central apparatus of motor coordination develops autonomously and precociously, in advance of demonstrable connection with sense organs or central sensory pathways. Sensori-motor arcs are closed later, and the first sensori-motor reactions so effected are not learned. The first movements in response to sensory excitation are made as soon as these central connections are effected and these are well-coordinated adaptive responses, employing a complex of motor adjustors which has matured in advance of this stage.

- (152) 79. *Early differentiation of the cells of the ovum in the rhesus monkey*. Chester H. Heuser, Department of Embryology, Carnegie Institution of Washington, Baltimore.

One of the blastomeres of the 2 celled stage of the monkey is slightly larger than the other and throughout the process of cleavage the cells in every ovum vary in size. The largest cell in general will be the next to divide. The segregation of materials which later develop into embryonic and extra-embryonic portions of the ovum occurs very early and the monkey ova correspond to similar stages of other mammals as regards the temporary inertness of the embryonic ('formative') cells. Recently acquired monkey embryos provide additional information concerning the behavior of the cells of the young blastocyst and its attachment to the maternal tissues. In a blastocyst measuring 0.175 mm. in diameter there are still a few cells at least double the size of others in the 'inner cell-mass.' The majority of the cells are in the process of forming extra-embryonic structures. This specimen further emphasizes the precocious differentiation of the trophoblastic portions of the ovum.

- (8) 80. *The regeneration of the enucleated adrenal gland.* G. M. Higgins and D. J. Ingle, The Mayo Foundation, University of Minnesota.

Enucleation of the adrenal gland in the rat consists in clipping off the tip of the gland and then by gentle pressure expressing the entire medulla and most of the cortex from the capsule. A thin layer of glomerulosa remains adherent to the capsule. A study has been made of the regeneration of such an enucleated adrenal as influenced by 1) presence or absence of an intact adrenal gland, 2) administration of cortin to the animal, 3) the removal of the pituitary body, 4) subsection of the animal to severe stress, and 5) age.

The following conclusions were reached. 1) Regeneration of an enucleated gland occurred rapidly when the other intact gland was removed. The presence of one normal gland inhibited the regeneration of the other enucleated one. 2) The oral administration of 5 cc. of cortin daily completely suppressed regeneration of the enucleated gland even when the other gland was removed. The subcutaneous administration of comparable amounts of cortin did not completely suppress regeneration. 3) Hypophysectomy completely suppressed regeneration of the enucleated adrenal. The adrenotrophic principle of the anterior lobe is essential for adrenal regeneration. 4) When the animal was subjected to unusual stress, as in the administration of carbontetrachloride, or of thyroxine, then extent of regeneration of the adrenal was greatly increased. 5) Age was not a factor in the regeneration of the enucleated adrenal.

- (93) 81. *Thyroid feeding and ovarian androgens.* R. T. Hill, Department of Anatomy, Indiana University, School of Medicine, Bloomington.

Castrated male mice had ovaries grafted into their ears, and were subjected to a thermal environment of 20°C., under which conditions the ovary assumes an androgenic function sufficient to maintain the accessories in a normal state (i.e., non-castrate male type). Under these conditions, the addition to the diet of a non-toxic amount of desiccated thyroid tissue will cause the regression of the accessories to the condition in a castrate. When the thyroid feeding is stopped the accessories may or may not return to normal, depending upon the relative amount of thyroid tissue which has been fed. In some instances they have not returned to normal in as long as 4 months. Certain experiments have been devised to test the site of action of the thyroid which was fed. These data suggest that the feeding of thyroid tissue does not affect the grafted ovary, but rather acts on the host organism, probably on the thyroid gland, and in some manner either inhibits or blocks the androgenic activity of the ovary.

- (193) 82. *The reaction of tissue culture cells to Trichomonas foetus.* Mary Jane Hogue, Department of Anatomy, University of Pennsylvania.

Tissue cultures from the amnion and intestine of 8-day chick embryos were grown on a medium containing chicken serum and modified Ringer's solution. *Trichomonas foetus* was grown in pure culture, i.e., without bacteria, on egg slants covered with the same liquid medium. When the trichomonas were introduced into the tissue cultures growing in hanging drops they produced marked changes in the tissue culture cells. The epithelium, fibroblasts and nerves were

all killed by the toxic substance given off by the trichomonas. The filtrates from different aged cultures of trichomonas were also toxic to the tissue culture cells. When cultures of trichomonas were heated for 15 minutes at 55°C. they affected the tissue culture cells in the same way as the living trichomonas.

- (88) 83. *The healing of uterine wounds in the mouse, with observations on the effect of estrogen.* Charles W. Hooker, Department of Anatomy, Yale University School of Medicine. (Introduced by Edgar Allen.)

When a horn of the mouse uterus is slit longitudinally throughout its length, this tubular organ opens flat into a more or less ribbon-like structure. The tubular nature of the organ is restored in the remarkably short period of 2 to 4 days and the wound is completely healed. Soon after the wound is made an epithelial covering appears over the entire uterus, the cut surface being covered by contributions from both the endometrial and serosal epithelia. Reconstruction of the tube appears to be accomplished by activity of the circular muscle layer. There is a thinning of this layer in the mesometrial region and apparently a lateral migration of muscle cells. In the lateral portions of the flattened uterus a connection is established between the circular muscle and the longitudinal muscle in such a manner that contraction of the circular muscle would bend the organ into a tubular shape. Approximation of the epithelium results in the production of a lumen and the circular muscle fills the gap, apparently by migration and hyperplasia. Pavement epithelium may be caught within the uterus or, less frequently, columnar epithelium is left on the outer surface in the area of the wound.

Five hundred I.U. hydroxy estrin benzoate 24 hours before injury retards healing somewhat. In treated animals the tunica propria in open portions of the uterus is markedly edematous, while in closed portions of the uterus this edema is much reduced.

All the animals in the experiment, a total of seventy, were ovariectomized, and the colchicine technique was employed.

- (102) 84. *The arterial pattern of the dorsum of the foot.* John Franklin Huber, Department of Anatomy, Temple University School of Medicine.

On 85 out of 104 feet dissected, the dorsalis pedis artery was of sufficient size and so placed that its pulsation would probably have been readily felt in an area bounded medially by the tendon of the extensor hallucis longus muscle, superiorly by the cruciate ligament, laterally by the tendons of the extensor digitorum longus muscle and inferiorly by the extensor hallucis brevis muscle. It was noted that this area can be located by one angle of an equilateral triangle, the other two angles of which are formed by the medial and lateral malleoli.

On ten feet in the series, the dorsalis pedis artery was so small that its pulsation could probably not have been felt and it is interesting that these ten instances were all bilateral (on the right and left feet of five cadavers). On three feet, this artery was definitely under cover of the extensor hallucis longus tendon and on the remaining six, it was under cover of the extensor digitorum longus tendons and the extensor hallucis brevis muscle.

The variations found in the complete arterial pattern of the dorsum of the foot will be presented with their percentage of incidence. In only two of the fifty-two cadavers, was the common textbook picture present in its detail on both the right and the left foot.

- (66) 85. *Anomalous pulmonary veins draining oxygenated blood into the systemic veins.* Charles W. Hughes, Department of Anatomy, Loyola University School of Medicine. (Introduced by T. T. Job.)

In routine examinations of the pulmonary systems of 350 cadavers in the gross anatomy laboratory during the past 5 years, large anomalous pulmonary veins were found in two cadavers. Case 1: a 55-year-old white male, a vein 1 cm. in diameter and 1 cm. in length extended from the right lung to the superior vena cava. Case 2: a 44-year-old white male, a vein 1.5 cm. in diameter and 3.5 cm. in length extended from the left lung to the left innominate vein. In both of these cases the cause of death was cardiac decompensation and post mortem examination revealed cardiac enlargement.

A complete review of the literature has been made and a consideration of the embryological development of these anomalies has been included.

- (131) 86. *The early development of the nervous system of Amblystoma studied by the colchicine technique. I. Medullary plate stages.* Cranford Hutchinson, Morris Biological Farm of The Wistar Institute.

This is the first report on a projected study of the prefunctional development of the Amblystoma nervous system with special reference to the factors which influence its growth. For this study a closely graded series of embryos was raised at a constant temperature. At various stages these embryos were immersed in a solution of colchicine for regular periods of from 4 to 24 hours in order to hold in metaphase all mitoses which occurred during these periods. A standard solution of colchicine cannot be used because the embryos react differently at different stages of development. In all cases controls from the same egg masses were raised at the same temperature and preserved at the same time.

Counts of mitotic figures show that there is a period of rapid growth just prior to the appearance of the medullary folds (Harrison stage 14). This gradually slows up as the folds rise and begin to approach one another. To find out whether any relationship exists between the proliferation rate of the plate and 'unterlage,' counts were made of the latter but the results were negative. Each appears to have a mitotic pattern of its own, the plate showing the highest rate of growth in the region of the future brain and the substrate giving the highest counts in the region where the somites will first appear.

- (10) 87. *Atrophy of the adrenal cortex in the rat produced by the administration of large amounts of cortin.* D. J. Ingle, G. M. Higgins and E. C. Kendall, The Mayo Foundation, University of Minnesota.

Large amounts of an active extract of the adrenal gland have been given to rats by mouth from 1 to 28 days. Likewise two active crystalline compounds, from the adrenal cortex, known as A and B were given orally each day for 1 week.

Both the total extract and the crystalline compounds produced extensive atrophy in the cortex. There was a marked relationship between the amounts of total extract administered and the extent of atrophy induced. Likewise there was a marked relationship between the time of administration and the extent of atrophy. In an attempt to determine the role of the anterior pituitary in the production of cortical atrophy, the hypophysis was completely removed from a series of rats. These were then given an adrenotropic fraction, prepared by Dr. H. D. Moon in amounts sufficient to prevent cortical atrophy which normally follows hypophysectomy. When such animals were administered the massive doses of cortin, cortical atrophy did not occur.

- (3) 88. *The nature of the abnormally rapid increase in body weight following a period of growth suppression.* C. M. Jackson, Department of Anatomy, University of Minnesota.

It is well known that in young animals a period of growth suppression is often followed by a transient abnormally rapid growth. To determine what organs or parts are responsible for this excessive growth rate, six groups of twenty-four young albino rats each were autopsied and their organ weights compared at three levels of body weight. 1) At about 52 gm. body weight, normal initial controls at 3 weeks of age (just weaned) compared with rats held at constant weight by underfeeding from 3 to 18 weeks of age. 2) Body weight doubled (104 gm.), normal control rats and refeed test rats. Time required for doubling weight, 9 days in the test rats and 15 days in the controls. 3) Body weight quadrupled (208 gm.), requiring 35 days in the test rats and 39 days in the controls.

The excessive early increase in the body weight of the test rats upon refeeding is not due solely to increased gastrointestinal contents or to excessive growth in any single organ or system. It is due rather to a generalized acceleration affecting many organs and parts, which show variable rates of increase. The previous abnormalities (characteristic results of the preceding dystrophic growth period) tend to become corrected, so that ultimately the various organs resume nearly normal proportions for corresponding body size.

- (21) 89. *The morphology of the thymus and its changes with age in the neotenus amphibian (Necturus maculosus).* Emory S. James, Washington Square College, New York University. (Introduced by Robert Chambers.)

The thymus consists of a pair of bodies on each dorsolateral surface of the head anterior to the gills. The anterior body is bounded by the masseter and sacrospinalis muscles medially; laterally by the ceratohyoid, while the levator branchiarum crosses its dorsal surface. The posterior body rests dorsal to the third efferent artery, lateral to the levator branchiarum, and medial to the epi-branchial cartilages.

The vertebral and pulmonary arteries supply the anterior body, the pulmonary sends a branch to the posterior body. The external jugular vein and accompanying lymph vessels drain the thymus and adjacent tissue. The glossopharyngeal-vagus trunk sends fibers to the capsule and parenchyma of the gland.

The thymus bodies are each enclosed by a thin connective tissue capsule from which trabeculae extend into the parenchyma. The cellular elements include small thymocytes or lymphocytes, larger lymphocytes, epithelial cells, connective tissue cells, large cells with reticular cytoplasm and excentric nuclei, and large granular eosinophilic cells. Other structures include Hassall's corpuscles, cysts, and follicular or alveolar formations.

The formed elements include loose and compact Golgi network, mitochondria and granules in the eosinophilic cells. A secretion cycle is described based on changes in the eosinophilic cells.

The glands of older animals exhibit changes in structure comparable to involution in other vertebrate forms.

- (170) 90. *Some effects of injection of hypophyseal extract on the ovaries of the bat.* Katharine R. Jeffers and Mary J. Guthrie, Department of Zoology, Duke University, and Department of Zoology, University of Missouri.

The authors have reported previously that female bats of the genus *Myotis* can be induced to ovulate during the winter by three daily, intra-peritoneal injections of 0.1 cc. each of hypophyseal extract provided by J. B. Collip. When the ovaries of twenty-two treated bats (*M. lucifugus lucifugus*), killed at intervals up to 10 days after the final injection, are compared with ovaries of uninjected bats, killed within 24 hours after collection, effects of the injections other than premature rupture of the single vesicular follicle can be detected. There is a conspicuous increase in the relative number of the smallest growing follicles. Growth and cytoplasmic differentiation of the oocyte occur more slowly, in relation to growth of the follicle, in injected bats. The secondary follicles grow beyond the size at which their degeneration normally begins, and small antra may be formed. The injections tend to inhibit the formation of meiotic spindles in mature oocytes of retrogressing follicles.

- (47) 91. *The distribution of efferent fibers in the internal branch of the superior laryngeal nerve.* T. T. Job and H. H. Todd, Jr., Department of Anatomy, Loyola University.

By minute dissections and a modification of the silver-chloride technic, we have been able to demonstrate a motor component in the internal branch of the superior laryngeal nerve, which innervates the aryepiglottic, oblique and transverse arytenoid muscles. This condition has been found in each of the eleven fresh human larynges examined. The larynges of ten dogs revealed a different arrangement of the musculature but a corresponding innervation.

An attempt has been made to cover the pertinent literature since the time of Galen. The suggestion of a dual innervation for some of the laryngeal muscles, usually accredited by Exner (1884), was made by Alexander Monro, secundus, in 1783.

- (38) 92. *The origin of sheath cells in the chick.* David S. Jones, Department of Anatomy, Loyola University School of Medicine. (Introduced by T. T. Job.)

The neural crest cells, together with varying amounts of the dorsal portion of the neural tube and overlying epithelium, were removed from chicks at approximately 45 hours of incubation. The operative field extended over several segments at the level of the vitelline vessels. Following the operation, the embryos were reincubated for 5 days. Microscopical examination of serial sections shows the region of the operation to be free of dorsal root ganglia. The ventral roots are well developed in regions where the neural tube is not severely damaged. These ventral roots are richly supplied with sheath cells.

Near the end of the wound in one specimen there is a spinal ganglion which is not connected with the spinal cord. An aberrant nerve extends laterally from the ganglion. This nerve is supplied with sheath cells. Thus sheath cells are present in nerves proceeding independently either from the spinal cord or from a spinal ganglion. If the sheath cells are of ectodermal origin, then both the spinal cord and spinal ganglia contain cells capable of differentiating into sheath cells. (See demonstration no. D29.)

- (108) 93. *The significance of megaloblasts.* Oliver P. Jones, Department of Anatomy, University of Buffalo.

Since European monographs have recently emphasized that megaloblasts are found almost exclusively in bone marrows of pernicious and related types of anemia, it seems appropriate to reiterate certain claims previously advanced (Jones, '34 and '35). Megaloblasts are the predominant type of red cell development in pernicious anemia marrow during relapse. The youngest megaloblasts are usually derived from the myeloblast and rarely from the reticulo-endothelium. The end products of this series are reticulated and mature megalocytes which are responsible for the increased color index, corpuscular diameter and mean corpuscular volume in the peripheral blood. Megaloblasts are not present in normal bone marrow and are therefore not related to normoblasts. Kirschbaum ('37) has shown that pernicious anemia megaloblasts are similar to the primitive erythroblasts of the embryonic rat yolk sac but not identical with them. Until fresh dry smears of human embryonic blood are compared with smears of pernicious anemia bone marrow and blood, megaloblasts should be considered separately as a pathologic red cell type. Confusion in this country regarding the true nature of the megaloblast is due to the fact that Doan, Cunningham and Sabin redefined the term indiscriminately to include primitive erythroblasts, pronormoblasts (proerythroblasts) and promegaloblasts. It is necessary to use dry smear or imprint preparations stained with May-Grünwald-Giemsa in order to see the finest detail of nuclear structure. (See demonstration no. D30.)

- (25) 94. *An experimental study of the function of the ligaments and muscles of the human foot, and their relation to the support of the arch.* Russell L. Jones, Department of Anatomy, Indiana University, School of Medicine. (Introduced by B. D. Myers.)

Muscles and ligaments are, obviously, both factors in the support of the arch, but great differences of opinion exist concerning the relative value of these factors. Opinions on this subject are largely deductions from observations of the attachments of muscles and ligaments. The problem is here approached by securing experimental data by means of appropriate apparatus.

Hooks were fastened to the long muscle tendons of a dissected leg and foot, which, singly and in combination, were subjected to known tensions in pounds, and the resulting actions, their effect upon arch support, and their effect upon the distribution of pressure at the ball of the foot, were recorded in pounds. The distribution of weight upon the various metatarsal heads was also determined in the experimental foot and in living feet both with and without support of the long muscles.

Indications are that the long foot muscles are quantitatively much less important to arch support than the plantar ligaments and muscles; that the main function of the long muscles excluding dorsiflexors and the gastrocnemius-soleus is the reflex equalization and adjustment of weight distribution upon the metatarsal heads comprising the ball of the foot; and that stress on the arch is approximately doubled when the weight is supported entirely on the ball of the foot.

Other data and the application of findings to flat-foot are discussed.

- (183) 95. *Smooth muscle simulacra of discs of cross-striated muscle.* H. E. Jordan, Department of Histology and Embryology, University of Virginia.

The muscle columns of the gizzard of young fowls can be completely dissociated by maceration in solutions of caustic potash into discrete typical fusiform smooth muscle cells. There remains no histologic basis for the interpretation of these muscle columns in the regions designated 'transitional musculature' and 'cross-striated' as 'fibers' enveloped by sarcolemma. Much of the muscle wall consists of spindle-shaped cells with smooth contour. In the area near the muscle-tendon junction the muscle columns have a closely cross-striped appearance. Distally the muscle columns show widely spaced bands, contraction nodes. Isolated cells from this region show individual contraction nodes. Isolated cells from the striped region show many undulations, zigzag foldings or even spiral deformations. The cross-stripes result from a precise horizontal alignment of the undulations by connective tissue fibrils. Cross-striped features of smooth muscle present simulacra of genuine cross-striated muscle, but signify only passive distortion, a longitudinal buckling of constituent cells, resulting from pressure of a contracting mass of muscle upon certain areas against the resistant barrier of the central tendon. The striations on the smooth muscle of the dog's bladder have the same significance. The histologic data give no basis for the claim that definitive smooth muscle may become transformed into the cross-striated variety. (See demonstration no. D31.)

- (117) 96. *Thalamic ending of the brachium conjunctivum.* Park D. Keller, Department of Anatomy, Cornell University, Ithaca. (Introduced by J. W. Papez.)

When the dentate nucleus was destroyed the brachium conjunctivum degenerated. Marchi preparations showed two streams of fibers into the thalamus. The division occurred at the red nucleus. One stream arched dorsolaterally into the center median and paracentral nuclei which it followed. More orally these fibers spread out in the thalamus lateral to the ventral medial nucleus and internal medullary lamina. The other stream, the larger one, continued orally in the medial tegmental field, H₁. In the oral half of the thalamus these fibers shifted dorsally and laterally into the same intermediate region occupied by the dorsal stream at this level. Lateral to the ventral medial nucleus they sprayed out laterally and dorsally through the extent of the ventral anterior nucleus.

Conclusions. The thalamic terminus of the brachium conjunctivum includes the region of the center median nucleus, the paracentral nucleus, and an extensive area lying in the intermediate region of the thalamus lateral to the ventral medial nucleus, extending orally and laterally into the ventral anterior nucleus. This region might be called the ventral intermediate nucleus.

- (141) 97. *Regeneration of gonad tubules following extirpation in a sea cucumber.* Frank R. Kille, Department of Zoology, Swarthmore College. (Introduced by B. H. Willier.)

The capacity of Holothurians to regenerate gonadal tissue as suggested by certain indirect evidence has been tested in *Thyone briareus* Lesueur by the a) extirpation of the gonad tubules, and b) extirpation of the tubules and the gonad basis from which they arise. No replacement follows the latter operation. When only the tubules are removed, new tubules may or may not be proliferated from the gonad basis. Histological evidence indicates that this variation is due to the fact that certain tissue of the gonad basis containing germ cells may or may not be removed with the tubules. That the proliferation of new tubules from this tissue may depend upon the presence of germ cells is indicated by 1) sterile stumps of the original tubules never produce new tubules, and 2) among the newly proliferated tubules, no sterile tubule has ever been found. Thus direct evidence is given that a restricted region of the gonad basis has the capacity to proliferate new tubules in numbers far greater than the normal seasonal additions if only the gonad tubules are removed. To speak of "regeneration of the Holothurian gonad" is misleading, the situation being analogous to that in vertebrates where growth of the accessory or residual gonad tissue may follow incomplete extirpations.

- (126) 98. *The central and peripheral development of the vagus and glossopharyngeal nerves in the rabbit embryo.* Donald L. Kimmel, Department of Anatomy, University of Michigan. (Introduced by Elizabeth C. Crosby.)

In early embryological stages of the rabbit medulla, vagus and glossopharyngeal nerves arise from an undifferentiated, subventricular gray. This gray, with that adjoining it rostrally and caudally, shows an increasing multipolarity of neurons,

and forms a discrete common visceral efferent column which differentiates in turn into a general visceral efferent column and a more ventrally placed special visceral efferent column (the nucleus ambiguus). Later a superior and an inferior salivatory nucleus and a dorsal efferent nucleus form from the visceral efferent column.

Neuraxes of sensory, bipolar ganglion cells are traceable to differentiating receptive nuclear groups after the thirteenth day. To such groups belong the gray of the fasciculus solitarius and of the descending trigeminal root, the nucleus praepositus, the nucleus intercalatus, and other small-celled nuclear masses contiguous to the hypoglossal nucleus.

Two ganglia per root are evident at the thirteenth, and are compact, encapsulated structures by the fifteenth day. By the twenty-fifth day their cells are mostly unipolar.

The peripheral development of these nerves occurs in the following order: 1) the appearance of peripheral branches; 2) the beginning of plexus formation in mesenchyme about the structures to be innervated; 3) the appearance of terminal autonomic ganglion cells; and 4) the invasion of the (thoracico-lumbar) sympathetic fibers into the various plexuses and anastomoses occurring between this sympathetic system and the root ganglia of these nerves.

- (107) 99. *A quantitative histologic study of the lymphoid organs of 20-day and 30-day-old albino rats.* J. E. Kindred, Laboratory of Histology and Embryology, University of Virginia Medical School.

Quantitative histologic analyses of submaxillary, mesenteric and inguinal lymph nodes, spleens, Peyer's patches and ceca of ten 20-day-old rats, and submaxillary nodes of nine 30-day-old rats form the basis of this study. Since secondary nodules are most constant in occurrence in the submaxillary nodes, these nodes of 20- and 30-day-old rats are compared. The secondary nodules increase in size and number and exhibit the following changes in cell populations and mitotic indices. Decrease in number of small lymphocytes and reticulum cells; increase in number of medium-sized, large and giant lymphocytes, and macrophages. Macrophage index changes from 28% to 50% regardless of size of nodule. The mitotic index for all cells remains about the same (2.1% and 2.5%), but there is a striking increase in the mitotic index of large lymphocytes from 7% to 26%; and practically all giant lymphocytes are in mitosis. The medullary cords show conspicuous reduction in small lymphocytes and reticulum cells; and great increase in medium-sized, large and giant lymphocytes, and plasma cells. Granulocytes characteristic of the 20-day stage have all but disappeared at the 30-day stage. The mitotic index for all cells in both ages is about the same (1.2% and 1.6%), and is distinctly greatest in the large and giant lymphocytes.

- (208) 100. *Studies on the lymphocytes of normal and leukemic mice.* A. Kirschbaum, F. J. Lits, L. C. Strong and W. U. Gardner, Department of Anatomy, Yale University School of Medicine.

Ten lines of transplantable lymphatic leukemia, a pure mediastinal lymphosarcoma (transplantable thymoma?) and a transplantable myelogenous leukemia were studied in four highly inbred strains of mice (F, A, C₃H and CBA). Large cells, many 'lymphoblastic' or 'myeloblastic,' characterize the lymphoid condition in the F mice. Maturation of immature forms to large and medium-sized extremely basophilic lymphocytes with coarse nuclear chromatin pattern is found in certain lines of the other strains. The latter cell types, as well as the more immature lymphocytes, divide by mitosis in the blood and tissues. Dry imprints of normal lymph nodes indicate that cells morphologically similar to 'malignant' forms are present, but do not predominate as in leukemic nodes.

Temporary regression of local lymphoid tumors at the implantation site (14-day growth in C₃H strain) was brought about by the subcutaneous administration of 1/40 mg. of colchicine every third day. Survival time was lengthened in treated mice, tumor-bearing animals serving as controls. (There were 'takes' in 100% of the animals inoculated, death resulting in all cases within 40 days if untreated. Systemic leukemia usually developed following the formation of a local tumor.) An increased incidence of spontaneous lymphatic leukemia manifested itself in certain strains of mice subjected to high doses of estrogens over a long period of time (Gardner). (See also abstract no. 114, and demonstrations nos. D33 and D39.)

- (110) 101. *The development of plasma cells from reticulo-endothelial cells as seen in successive bone marrow biopsies in the rabbit.* Fred Kolouch, Jr., Department of Anatomy, University of Minnesota. (Introduced by Hal Downey.)

The presence of an increased number of plasma cells in the bone marrow of a patient with a fatal infectious disease (bacterial endocarditis), indicated a possible correlation between the infectious process and the development of plasma cells.

Rabbits were made allergic by a subcutaneous injection of *Streptococcus viridans*. After allergy was produced, an intravenous injection of the same organism had a stimulating effect upon the reticulo-endothelial system.

The initial proliferation is followed by a metamorphosis of the cells. The leptochromatic nuclei show a progressive condensation of their chromatin accompanied by contraction which results in the pachychromatic nuclei characteristic of plasma cells. The foamy mildly basophilic cytoplasm of the reticulo-endothelial cells undergoes a decrease in volume accompanied by progressively increasing basophilia. Concurrently the cells round up and the hof appears.

The transition of the reticulo-endothelial cells to plasma cells can be followed only through successive biopsies since it is a cursory phenomenon. The allergic state is probably essential in this process. (See demonstration no. D35.)

- (180) 102. *Cellular specialization in ciliated epithelium.* S. I. Kornhauser, Department of Anatomy, University of Louisville.

Cytological observations on the ciliated cells of the gills of *Nucula*, a primitive lamellibranch, and *Ostrea*, a highly specialized form, reveals that in the lateral cells there is a tendency of the cellular elements to become elongated in the direction of the conduction of the wave even though this is at right angles to the effective beat of the cilia. The epithelial cells instead of being hexagonal in surface view become quadrangular and arranged in perfectly straight rows in the direction of conduction. The nuclei likewise elongate with the cell bodies. *Nucula* already shows some tendency in this direction. In *Ostrea* it is completed. For the movement of water, respiratory currents, a regular arrangement of the cilia in rows 45° to the long axis of the cells forming a perfect criss-cross surface pattern occurs in *Nucula*. In *Ostrea*, the basal bodies of the cilia assemble in single complete diagonal rows 45° to the long axis of the quadrangular cells. For the cells which move food particles and mucus, namely the frontal ciliated epithelium, no such regularity of cilia or cell rows occurs. In the laterofrontal cells we find a highly specialized apparatus of fused cilia and elastic rod used to divert food particles from the lateral cells and direct them toward the frontal cells.

- (103) 103. *Histo-physics of the aorta.* Joseph Krafka, Jr., University of Georgia School of Medicine.

In support of the theory of intimal herniation as the principle mechanical factor in the etiology of arteriosclerosis, tests were made on Young's modulus for the ligamentum nuchae and the aorta where test strips were taken from the same animal and subjected to stretch in a Scott's serigraph. Average value of M^{100} for ligamentum nuchae is 4.60×10^6 ; for the aorta, 1.15×10^6 . Elasticity in the aorta is a function of the net-like arrangement of fibers rather than the physical property of elastic tissue per se.

- (91) 104. *Roentgen studies of the mechanism involved in sperm transportation in the female rabbit.* Robert H. Krehbiel and Herman P. Carstens, Departments of Anatomy and Physiology, University of Illinois.

Iodochlorol placed in the vagina of isolated virgin rabbits has been observed, with the aid of the fluoroscope, in its progress through the cervix and uterus after artificial stimulation of the vulva. Erection of the vulvar tissue, in animals in heat, occurred in less than 1 minute after the beginning of stimulation. Concomitant spiral contractions of the vagina, and the appearance of iodochlorol in the cervix were noted. In each positive instance the oil was seen in the ovarian end of the uterine horns within less than 5 minutes after the start of stimulation. Observations were continued over a period of an hour. In no case was the oil seen in the oviducts. Similar results were obtained with the use of methylene blue in animals under local or general anesthesia. Uterine contractions in the visible portions indicate that the transfer from vagina to uterus is dependent upon the combined activity of both organs. Progress through the uterine horns is due to peristaltic contractions starting from the ovarian end of the uterus and moving toward the cervical end.

- (118) 105. *Effects of experimental hypothalamic lesions.* Wendell J. S. Krieg, Department of Anatomy, College of Medicine, New York University.

The hypothalamus of the albino rat was subjected to irritation by the pressure of mercury in the third ventricle, or was partially destroyed by electric cauterization. The animals were kept under observation for a number of days. The various animals presented one of the following syndromes: poikilothermy; an emotionally hyperactive state manifesting itself as rage, attack or flight on slight stimuli; pupillary dilatation; exophthalmos; depression; alterations of vascular tonus; alterations of feeding reactions. Finally the animals were killed, the brains sectioned serially, and the lesions localized in terms of nuclei and fiber tracts.

- (191) 106. *The mineral content of human amnion and chorion at term as studied by micro-incineration.* Benjamin Kropp, Departments of Anatomy and Obstetrics, Harvard Medical School.

The fused chorionic and amniotic membranes, together with the intermediate connective tissue, were studied for heat-resistant ash residues by the micro-incineration method. The tapering columnar epithelium of the amnion is rich in calcium, iron and water-soluble minerals. Most of the minerals are contained in the cytoplasm where they appear as an evenly distributed, powdery residue, with only occasional coarse whitish granules. Vacuoles of the cytoplasm are essentially ash free. The nuclei are outlined by white ash particles, insoluble in water, and are, for the most part, poor in mineral ash. In localized areas the amniotic epithelial cells show at their outer or lateral margins increased amounts of iron oxide and water-soluble ash.

The cytoplasm of the chorionic epithelium is less rich in total mineral ash, but relatively richer than amnion in calcium and insoluble ash residue. The nuclei appear invariably as almost ash-free areas surrounded by a fine white granular ash.

The interposed loose, gelatinous connective tissue contains an abundance of white mineral ash very slightly soluble in water. Standing out conspicuously by contrast are a few areas containing very little mineral ash. The latter are believed to represent the mineral poor elastic fibers, while the mineral rich portions represent mainly the collagenous bundles. This connective tissue contains scattered cells that are moderately rich in iron oxide.

- (114) 107. *The ontogenetic development and cytoarchitecture of the pretectal nuclei in the chick.* Hartwig Kuhlenbeck, Department of Anatomy, Woman's Medical College of Pennsylvania.

During the fifth and sixth day of incubation the four longitudinal diencephalic zones are still relatively undifferentiated cell columns exhibiting the primitive vertebrate pattern. At this stage the cell masses of the epithalamus end at the rostral boundary of the posterior commissure. The thalamus dorsalis extends caudad beneath the posterior commissure and accompanies this structure to its caudal boundary. Caudalward the thalamus dorsalis is limited by the primordia of the tectal formation and the torus semicircularis. Basalward the caudal portion of the thalamus dorsalis reaches the sulcus limitans and adjoins the tegmental cell cord.

The subsequent differentiation of the pretectal nuclei occurs within this caudal portion of the dorsal thalamic zone coextensive with and closely related to the posterior commissure.

The pretectal cell masses in the fully developed brain can be subdivided in three major groups: 1) nucleus principalis praecommissuralis, 2) main pretectal group with several subdivisions, 3) several nuclei of the posterior commissure. The nuclei of these three groups as well as the posterior commissure must be considered to be essentially diencephalic structures.

Associated with these nuclei are two nuclei of tectal origin, nucleus lentiformis mesencephali and nucleus parageniculatus tecti and furthermore one tegmental structure, nucleus interstitialis tegmentalis commissurae posterioris.

A total of more than fourteen different cytoarchitectural units can be distinguished among the pretectal cell masses in the chick.

- (50) 108. *Afferent innervation of the skin.* Albert Kuntz and John W. Hamilton, St. Louis University School of Medicine.

Observations on preparations of normally innervated human and animal skin and skin of the forelimbs of cats in which the sympathetic fibers had undergone degeneration following sympathectomy support the current view that afferent nerve fibers terminate in the deeper layers of the epidermis and throughout the corium. Many of the smaller afferent fibers terminate in relation to the cutaneous blood vessels, including the capillaries, particularly in the papillary layer of the corium. This anatomical relationship seems to support the assumption that the papillary vessels play a functional role in cutaneous sensitivity.

- (62) 109. *A quantitative study of the gray and white matter of human fetal spinal cords.* A. M. Lassek and G. L. Rasmussen, Department of Anatomy, Medical College of the State of South Carolina.

The regional growth changes occurring in the gray and white matter were studied in a series of nineteen fetal spinal cords in the Negro. Between the fourth and tenth months of fetal life, the spinal cord increases in length 2.7 times, gray area 2.7, white area 4.5, gray volume 6.6, white volume 14.2, and in total volume 10.0 times. The thoracic length augments at the expense of the lumbar and sacral divisions. The sacral gray area and volume show the greatest relative increases while the thoracic white area and volume have the greatest proportionate increments. The gray matter develops two times, the white matter four, and the entire substance three times as fast in actual volume during the latter half of gestation as during the first half. At all times during prenatal life, the percentages of the regional lengths and gray volumes as compared to the totals are about the same while the white substance percentages vary as follows: the cervical white remains the same, the thoracic increases, and the sacral and lumbar decrease.

(30) 110. *Weights of the hypophysis, thyroid, suprarenals and gonads in the cat.*

Homer B. Latimer, Department of Anatomy, University of Kansas.

The weights of these four glands were determined in 229 fetal, 35 newborn and 104 adult cats.

The weights of the ovaries form a straight line curve when plotted on body weight and the others all increase more rapidly at first, gradually decreasing in rate of growth. Plotted on body length, the testes form a straight line curve and all of the others form curves concave superiorly.

The percentage weights of all of the glands decrease during the fetal period and all except the testes decrease between birth and maturity.

In the adult cats, all of these glands, except the hypophysis, are more variable in weight in the males. The hypophysis is the most constant in weight and the gonads the most variable in both sexes.

Correlations between body weight and the weights of these glands are positive in all cases and significant for all of the male glands except the hypophysis. In the females, only the gonads and the suprarenals form significant positive correlations with the body weight. The correlations of the hypophysis and the thyroid are positive but not significant.

The correlations between the weights of the hypophysis and the other glands in the males are positive but not significant. In the females, the similar correlations are higher in every case, but only one, namely that for the suprarenals, is significant.

(48) 111. *Studies on the sensory structures of the ground mole (Scalopus aquaticus, L.). I. The epidermis of the nostrils and vibrissae of the snout.*

Sister Anna Catherine Lavior, Catholic University and College of Saint Elizabeth. (Introduced by Harry A. Charipper.)

The epithelium of the nostrils contains specialized regions known as sensory cups. Terminal nerve fibers from the maxillary branch of the trigeminal nerve ramify through the sensory cup, entering its base and sides by way of tactile cells. Merkel's tactile menisci are also found. The cup also contains numerous tactile corpuscles innervated from nerve bundles in the cup. Palisade fibers are present, passing through the germinativum to end freely in the lucidum. Neurofibrillar networks are found in contact with nuclei of certain of the sensory cells indicating intracellular nerve endings and continuity of nerve fibers. Rows of tactile hairs are found on epidermal ridges of the snout. They are most numerous in the first row just back of the nostrils. The presence of a venous sinus, 'Ringwulst' and a three-layered dermal sheath identify the tactile from the non-tactile hair. Palisade fibers, from two sensory trunks of the infraorbital nerve, are present over the entire surface of the hair follicle, and end in neurofibrillar networks around the nuclei of Merkel's tactile cells. The nerve fibers forming a nerve ring around the neck of the follicle, and others supply the outer layer of the dermal sheath. Golgi material and varied mitochondria are present in the sensory cup cells with epithelio-fibrillae most pronounced in the nostril basal epithelial cells.

- (177) 112. *A comparison of the pituitary complex in two morphologically different varieties of the goldfish (Carassius auratus) with special reference to the cytology.* Irving Levenstein, Washington Square College, New York University. (Introduced by Ruth B. Howland.)

The pituitary of the 'common' goldfish was studied and compared with that of the 'telescope moor.' These forms represent extremes in morphological development of *Carassius auratus*. The differences involve body shape, fin variations and color. The pituitary of the 'common' variety is larger than that in a comparable 'moor.' This difference may be related to the arrangement of the bony structures. The transitional lobe in the 'moor' contains (after Masson's) more heavily granulated and intensely staining eosinophiles than does that lobe in the 'common.' Differences in numbers and distribution of chromophiles exist in comparable sections of the gland. Confirmation of these findings will depend upon analysis of the cell counts. The Golgi configuration is similar in corresponding cells of both varieties. No differences are apparent in the intermedia of the black pigmented 'moor' as against the gold 'common.' Gross relationships of the gland and morphological divisions (Bell, '37) were confirmed for the 'common' variety and there appears to be no apparent variations of these parts in the 'moor.' The Golgi network of the intermedia cells completely surrounds the nucleus. A compact Golgi caps the transitional lobe eosinophiles. In the basophiles the network touches the nucleus on one side but ramifies out into the cytoplasm. In the chromophobes both types of Golgi are found. This condition is strikingly similar to that found in the mammals (Severinghaus, '32).

- (205) 113. *Lymphocytes and monocytes in tissue cultures of lymph nodes.* Warren H. Lewis, Department of Embryology, Carnegie Institution of Washington, Baltimore.

Many lymphocytes and small monocytes migrate out from lymph node explants during the first day. The lymphocytes and small monocytes are about the same size and at first glance one might easily imagine that all sorts of transitions exist between the two. Continued examination reveals, however, that the two types of cells are distinct and never have I been able to follow a transformation of a lymphocyte into a monocyte. Lymphocytes sometimes divide but they rarely survive more than a few days. The monocytes increase in size and multiply and survive for days.

The two types of cells can also be distinguished by peculiarities in their mode of locomotion. The same fundamental principles of locomotion apply to both types of cells and to other types as well. The distinguishing peculiarities are as distinctive as the morphology and are undoubtedly based on structural and physiological differences that are invisible.

Both lymphocytes and monocytes are probably independent self perpetuating strains of cells. (See motion picture demonstration no. D37.)

- (187) 114. *The effect of colchicine on normal and pathologic tissues of mice.*
F. J. Lits and A. Kirschbaum, Department of Pathology, University of Brussels
and Department of Anatomy, Yale University School of Medicine.

After administration of colchicine, a 'caryoclastic agent,' waves of mitoses and pyknoses are found in the thymus and lymphoid organs. An increase in the number of mitoses, followed by excretion of 'nucleoid spherules,' is also seen in the crypts of Lieberkühn. Cells having an "apparent or latent blastic potentiality" exhibit mitoses (reticulo-endothelial cells, germinal cells of epithelial layers, hair-follicle cells, parenchymal cells of several organs, tumor cells). These mitoses show characteristic changes. Their course is arrested in the metaphase, cellular membranes being swollen, cytoplasm clear, chromosomes clumped. Most of these cells in mitosis die, very few mitoses going on to completion. Similar effects have been demonstrated in cold-blooded animals and plants. New growths (scars, granulomas, benign and malignant tumors) are very sensitive to the 'radiomimetic' properties of colchicine.

The study of colchicine-effect on a malignant lymphoid growth (see abstract no. 100) was particularly interesting, since it had previously been shown that normal lymphoid tissues are extremely sensitive. One-fortieth milligram doses result in the appearance of numerous mitoses, pyknosis of mitotic figures and pyknotic nuclei in quiescent cells. Repeated treatment causes a primary regression (nearly complete) of the tumor, but some cells which are probably resistant remain. Test animals live longer than controls. These results further suggest the 'radiomimetism' of caryoclastic agents. (See also abstract no. 100 and demonstrations nos. D33 and D39.)

- (192) 115. *Effects of intranuclear inclusions of virus origin on the nuclear substances.* Alfred M. Lucas, Department of Zoology, Iowa State College.

Intranuclear inclusions produced in brain meninges cells by fox encephalitis virus cause, in addition to the usual basichromatin margination, an extraction of oxychromatin from the basichromatin which substance is then moved centrally toward the inclusion body. The oxychromatin is negative to Feulgen's thymonucleic acid test. The intranuclear inclusion is positive from its first appearance. The marginated basichromatin breaks up into small granules, these become small spheres and finally larger masses. The nucleus shrinks and the nuclear sap is lost. These autolytic changes do not affect the appearance of the inclusion body.

Nuclei of monocytes and giant cells which contain inclusions produced by the submaxillary gland virus of guinea pigs, undergo liquefaction of chromatin during autolysis but the shrinkage phase is slight. Inclusions, free of their nuclei, have been observed in the cytosome of phagocytic cells, which suggests their resistance to nuclear autolysis.

Complete autolysis has not yet been observed in submaxillary gland duct cells which contain intranuclear inclusions. The process of margination of basichromatin is approximately the same as in monocytes. Later the basichromatin moves centrally to form a thin shell around the inclusion body. The breaking of this shell into larger aggregated masses of basichromatin suggests nuclear autolysis but other evidences of nuclear deterioration are lacking.

- (31) 116. *The origin and significance of the marsupial pouch.* Edward McCrady, Jr., The University of the South, Sewanee.

In the opossum the earliest anlagen of scrotum and pouch are hidden from external view beneath the thick epitrichium. In sections they are seen in both cases to be paired folds immediately anterolateral to the phallus. In position the scrotal folds in the male correspond to the posterior end of the pouch folds in the female. As in the female the posterior ends of the marsupial folds approach each other and fuse in the midline to form the posterior lip of the pouch just cranial to the clitoris; so in the male the scrotal folds fuse to form a medial scrotum just cranial to the penis. Later both pouch and scrotum migrate a considerable distance cranial from the clitoris and penis, respectively. The attachment of the inguinal ligament is the same in both sexes. In *Chironectes* the adult male has a pouch; and, according to Enders, the contraction of the cremaster muscle pulls the scrotum up to "the ventral body wall in the region forming the dorsal surface of the pouch." All of these facts point to the probable homology of the scrotum and the posterior portion of the marsupial folds. This would mean that the original anlagen of both are homologous with the Eutherian genital swellings, and that in spite of eventual anterior displacement, the lips of the pouch represent the Eutherian labia majora.

- (67) 117. *Observations on epicytes of the alveolar wall of the cat's lung, and their reactions when stimulated with osmium tetroxide.* Charles C. Macklin, University of Western Ontario.

By passing air, which has been bubbled through a 1% solution of osmium tetroxide in distilled water, into the right lower lobe of the living anesthetized cat's lung via a no. 12 French urethral catheter, a reaction of the elements on the alveolar walls of the vicinity was set up. This included the epicytes (also called septal cells, and other names). The previously small clear droplets of the cytoplasm became much enlarged, and there was an increase in the size of the cell. Often the cytoplasm seemed to be extended as a thin sheet over adjoining territory. Mitotic figures were numerous. Many cells became free in the alveolar cavities. Later on, the alveolar walls became covered with cells, often resembling an epithelial layer. Great mitotic activity was noted in the reacting bronchiolar and bronchial epithelium of the region. Dosages of varying intensity were used. The various fixatives were injected into the bronchial tree, or the blood vessels. Frozen sections, flattened against the slide, were particularly useful in permitting en face inspections. The usual thin sections, and also thick sections, were used after various modes of staining. (See demonstration no. D41.)

- (56) 118. *Activation of heat loss mechanisms by local heating of the brain.* H. W. Magoun, Institute of Neurology, Northwestern University Medical School.

Local heating of the brain of the cat with low voltage, high frequency current passing between electrodes oriented with the Horsley-Clarke apparatus, has demonstrated a reactive region which responds to an elevation of temperature by initiating heat loss activities, with marked acceleration of respiration, panting and the appearance of sweat on the foot pads.

The reactive elements appear to be concentrated in the medial portion of the caudal part of the ventral telencephalon, and, in lesser concentration, are continued backward through the diencephalon as far as the anterior end of the midbrain. In the telencephalon the responsive region occupies a position between the anterior commissure and the base of the brain. Through the diencephalon it is located in the hypothalamus and the ventral part of the thalamus, and occupies a progressively more dorsal position at more caudal levels. At the anterior end of the midbrain it is located in the vicinity of the central gray matter around the aqueduct.

The results are interpreted as indicating that the reactive region contains structures which are activated by the rising temperature of the blood and lead to heat loss activity in the normal animal when overheated.

- (85) 119. *Relation of uterine regression to menstrual bleeding in intraocular endometrial transplants in the rhesus monkey.* J. E. Markee, Department of Anatomy, Stanford University.

The withdrawal of estrin or progestin causes regression and thinning of the endometrium. The resulting disproportion between the thickness of the endometrium and the length of the coiled arteries appears to cause end to end compression of them, since as many as eight new coils may appear in the 24 hours preceding menstruation and since the distance between the ends of the arteries and the uterine epithelium may be halved in that interval. The increased coiling of the arteries appears to slow the circulation in the functional zone and the relative stasis and variable vasodilatation appears to cause leucocytosis and degeneration of the stroma and walls of the branches of the coiled arteries. When the weakened walls give way, menstrual hemorrhages occur which are terminated by constriction of the portion of the coiled arteries just outside the necrotic area. This vasoconstriction begins 4 to 24 hours before the onset of menstruation and persists throughout the period except when hemorrhage occurs from a branch of a coiled artery or one of the veins. Since the straight arteries in the basal zone are not affected by the regression, the basal zone, from which regeneration subsequently occurs, receives an unaltered blood supply throughout the menstrual period.

- (68) 120. *The measurement of pH in circulating blood.* Clyde Marshall and Leslie F. Nims, Laboratories of Neuro-Anatomy and Physiology, Yale University School of Medicine.

The Burr, Lane, Nims microvoltmeter originally designed for the measurement of bioelectric potentials combines high sensitivity and stability and is peculiarly suited for the study of pH in living systems. A modification of the instrument, together with the necessary apparatus for the measurement of the pH of circulating blood is described. A specially constructed glass electrode, together with a silver-silver chloride reference electrode, is put into a small cannula which in turn is inserted into the blood stream. A potentiometer is interposed in the electrical circuit between the electrodes and the microvoltmeter. A continuous graphic record of the changes in pH is secured with either a photoelectric recording galvanometer, or a standard galvanometer and a suitable camera.

The apparatus described has been employed in a number of experiments on respiration and blood pH. Small pH changes with variations in normal breathing are shown. The time relationships of the acid changes following the rebreathing of CO₂, and the alkaline reactions of overventilation are described. A phenomenon of overshooting in returning to the base line after an experimental displacement of pH is noted. The accompanying blood pressure changes are shown.

- (157) *121. Placental and mammary transmission of vitamin E.* Karl E. Mason and W. L. Bryan, Department of Anatomy, Vanderbilt University School of Medicine.

Breeding rats reared and maintained on three different diets—A) commercial dog chow; B) commercial dog chow 80%, wheat germ 20%; C) purified E-deficient diet supplemented with 6 drops wheat germ oil, daily—were given an E-deficient diet during the last half of lactation. So limited was the placental and mammary transfer of vitamin E, and its storage by the suckling young, that eighty female offspring reared on the E-deficient diet and successfully mated at an average age of 65 days have consistently exhibited severe resorptions of E-deficiency during their first gestation. Such animals, properly controlled, can be used for E-assay tests during their first pregnancy. This elimination of 'first-litter fertility,' and dispensability of a proven resorption, reduces the time and cost of preparing animals for assay to more than one-half that heretofore required.

Seventy-two male offspring were maintained on the E-deficient diet for variable periods. Those from mothers on diet A showed very uniform testicular degeneration which began between the fiftieth and sixtieth days of age, coincident with the first appearance of mature spermatozoa. Those from mothers on diets B and C exhibited equally uniform and consistent testis changes 15 and 20 days later, respectively. The prophylactic effect on the male affords a particularly delicate measure of the placental and mammary transfer of vitamin E.

- (59) *122. Physiological and anatomical consequences of forebrain ablation in the teleost, Holocentrus.* Ralph G. Meader, Department of Anatomy, Section of Neuro-Anatomy, Yale University School of Medicine.

Removal of the forebrain of *Holocentrus ascensionis* is followed by no readily perceptible alteration in the normal behavior. No overt evidence of olfactory deficit appears which is perhaps to be expected in view of the relative unimportance of olfaction as compared with vision in the normal activity of the fish. Reactions to visual stimuli expressed in feeding behavior and responses to other external objects are elicited with the usual ease and exhibit the customary pattern. The characteristic diurnal inactivity of *Holocentrus* and its naturally individualistic social behavior make difficult the evaluation of a possibly higher threshold to external stimuli or a loss of the schooling reaction noted in decerebrate teleosts by other investigators.

Anatomical studies of the brains of animals which lived 54 to 61 days post-operatively reveal the presence of degenerated and normal myelinated fibers in the tracts relating the forebrain to more caudal regions. The forebrain of *Holocentrus* is connected by afferent and efferent medullated neurones to the deep fiber layer of the optic tectum, to the thalamus, and to the hypothalamus. An analysis of the chief systems of fiber tracts is presented, together with a description of an unusual nuclear mass, the nucleus prethalamicus.

- (54) 123. *The effect of fever upon the barrier between the blood and cerebrospinal fluid of children.* Cecilia C. Mettler, Claude McK. Burpee and Fred A. Mettler, Departments of Anatomy and Pediatrics, University of Georgia School of Medicine.

We have previously reported upon the study of the activity of the blood-cerebrospinal fluid barrier of normal children (Psychiatric Quarterly, October, 1937, and American Physiological Society, 1938) as measured by the Walter-Katzenelbogen test. It has been pointed out that the conventionally accepted figures for the bromide permeability quotient are insufficiently corrected for age and race and was concluded that 1) the Negro child exhibits a distinct tendency to easily pass bromide from the blood into the cerebrospinal fluid and 2) the permeability threshold of the blood-cerebrospinal fluid barrier is lower in the first 2 years of life than later. Another factor influencing the bromide permeability quotient is now brought forward in this paper. It has been found that febrility tends to decrease the resistance of the barrier between the blood and cerebrospinal fluid. While a greater amount of bromide passed the barrier in children recently exhibiting a febrile reaction than in those who had not recently experienced fever, no correlation between the intensity or duration of the febrile reaction and barrier function could be observed. The measure of the absolute unit of febrile reaction used in this report is expressed as a constant equal to the number of days of fever (above 100°F.) multiplied by the number of degrees above 100 exhibited at the time of the febrile fastigium.

- (53) 124. *The escape of particulate matter from the subarachnoid space in the dog.* Fred A. Mettler and Lane Allen, Department of Anatomy, University of Georgia School of Medicine.

The material presented is not novel but represents a departure from commonly held opinions that while solutions of salts escape readily from the subarachnoid space, large molecules do so with difficulty and particulate matter not at all. Early work by Key and Retzius to the effect that particulate matter does escape has been deprecated, due to the assumption that abnormal pressures were employed by these workers. They postulated a stomata-like arrangement (similar to that recently emphasized by Allen for the lymphatics of the diaphragm) in the Pacchionian granulations, thus affording a mechanism for the escape of particles into the superior longitudinal sinus. The authors have verified the escape of particulate matter as represented by ink into the superior longitudinal sinus when pressures were kept well within normal limits. Further, Hydrokollag 300 and ink rapidly leave the subarachnoid space via the olfactory nerve sheaths and are drained off into the deep cervical lymph nodes by means of a definite plexiform arrangement about the nerve sheaths communicating freely with the lymphatic channels. This work was also anticipated by Retzius but denied by others upon pressure-relationship grounds. The possibility of rupture in our studies has been precluded by the use of physiologic pressures and checked in the living animal without pressure disturbances. Furthermore careful histological study has failed to reveal any evidence of possibly ruptured barriers.

- (101) 125. *The blood supply of the spleen and its collateral circulation.* N. A. Michels, Daniel Baugh Institute of Anatomy, Jefferson Medical College.

In 100 cadaver dissections the lienal arterial system was never the same. Arteries entering the spleen varied from 7 to 35 (average 15). Divisional patterns comprise 1) Magistral type with long splenic trunk dividing near spleen, large, few branches (2 to 4), compact hilus and even borders. 2) Distributed type with short splenic trunk dividing far away from spleen, small, numerous branches (6 to 13), distributed hilus, notched borders. Branches of the splenic artery comprise 1) arteria terminalis, superior, inferior and media with their ultimate and preultimate branches (2 to 12); arteria polaris superior or its early counterpart the ramus oesophago-gastricus posterior ascendens; 3) inferior polar arteries (1 to 3) from the splenic trunk, its terminal branches or left gastro-epiploic; 4) short gastrics which have twenty-five different sites of origin, patterns of 2-4-6-7-10 being common; 5) pancreatic rami, a) short, b) a. pancreatica superior, c) a. pancreatica magna, d) a. caudae pancreatis, e) rami epiploici posteriores.

A. Collateral pathways uniting splenic branches with themselves comprise 1) outer and inner hilus transversals, 2) short transpancreatic, 3) anastomosis in loops of a tortuous splenic trunk. B. Collateral pathways uniting the system of the splenic artery with neighboring arteries comprise 1) arcus arteriosus ventriculi inferior, 2) circulus coronarius inferior, 3) circulus pancreatoduodenalis, 4) circulus gastro-lienalis accessorius, 5) circulus transpancreaticus longus, 6) arcus epiploicus magnus, situated usually in the posterior layer of the great omentum below the transverse colon and serving also as a pathway for the liver and stomach. Course, segments, tortuosity and index length of the splenic artery, and types of coeliac axis will be discussed. (See demonstration no. D43.)

- (94) 126. *Effectiveness of sex hormones administered as skin ointments.* Carl R. Moore, Jule K. Lamar and Naomi Beck, Hull Zoological Laboratory, The University of Chicago.

Testosterone and testosterone propionate applied on the skin in a lanolin-like menstrum (courtesy Doctors Stragnell, Schwenk and Gilbert) are highly effective in castrated males. Following castration twenty daily applications of propionate (0.6 mg.) on a 2-square inch shaved area on a rat, produces seminal vesicles larger than normal controls, and seven times the weight of untreated castrate controls. Ointment without shaving is effective. Untreated castrates absorb sufficient material from contact with treated cage mates to markedly stimulate reproductive accessories. Two daily applications to castrates, days 50 to 70, maintain seminal vesicle growth above normal controls. Normal males treated from day 22 to 37, killed day 38, show testis weights 45% to 70% below untreated litter mates. Treatment of females from day 13 to 19 of pregnancy induces death and resorption of embryos. Castrated guinea pigs produce coagulable ejaculates by the seventh day of ointment treatment.

Estradiol-containing ointment (commercial preparation) recommended for removal of wrinkles from women induces cornified vaginal smears in spayed rats after two applications of one-fifth recommended daily dose. Applications on the neck of normal male guinea pigs stimulates marked growth of mammary glands.

These observations emphasize the readiness with which sex hormones are absorbed through the skin and bring into question the alleged innocuousness of hormone-containing face creams for indiscriminate use by normal women.

- (112) 127. *The absorption of thorium dioxide by the reticulo-endothelial system of the dog.* Otto A. Mortensen and Russell L. Guest, Department of Anatomy, University of Wisconsin.

One hundred seventy cubic centimeters of radio-opaque, colloidal, ThO_2 (Thoro-trast) was injected intravenously into a young dog over a period of a year. The liver, spleen, lymph nodes and bone marrow were visualized on roentgenograms taken of the living animal. Microscopic sections of selected tissues revealed fixation of the ThO_2 largely within the cells of the reticulo-endothelial system and in the histiocytes of the supporting framework of the lung, kidney, gall bladder, appendix, choroid plexus, thyroid, submaxillary and adrenal glands. The glomerular epithelium of the kidney is rather uniformly infiltrated.

The distribution of the injected material in the bone marrow roughly parallels the distribution of red marrow. The marrow of the lumbar and thoracic vertebrae, limb girdles and proximal bones of the extremities is strikingly opaque, that of the caudal vertebrae and distal limb bones is not visualized on the x-ray films. An interesting time factor is illustrated in the marrow distribution. Nine weeks elapsed between the last injection and the autopsy. The marrow proliferated during this interval was free of thorium dioxide. Such material has been suggested (Huggins) as suitable for studies of marrow growth.

The radio-activity of thorium was demonstrated by exposing an x-ray negative to pieces of the dried liver and spleen. Fogging of the film was demonstrable after an exposure of 4 hours. (See demonstration no. D44.)

- (189) 128. *The innervation of the pancreas in the cat.* Russell L. Moseley, Department of Anatomy, University of Minnesota. (Introduced by A. T. Rasmussen.)

In the pancreas of the kitten and adult cat are found groups of neurons, which show, in preparations stained by the Cajal and Bodian methods, the typical appearance of autonomic ganglia. The cells are multipolar; are of a size similar to that of cells of the inferior mesenteric and pelvic plexuses; possess a neurofibrillar structure, Nissl bodies, a pericellular plexus, and a large vesicular nucleus; are surrounded by satellite cells which are distinctly different from insular cells. The ganglia, containing neurons whose number ranges from twenty, in a single cross section, to one cell lying along a nerve trunk, are found in the interlobular spaces, well enclosed by connective tissue, and rarely in close relationship to insular or acinar tissue. The ganglia lie along nerve trunks which contain both myelinated and non-myelinated fibers, and which can be traced up to and through several successive ganglia. The smaller nerve trunks leaving a ganglion, which are predominantly non-myelinated, with the exception of the large nerves ending in Pacinian corpuscles, can be traced along interlobular ducts and arterioles, into the lobules of acinar tissue, where they lie beside, and accompany the intralobular capillaries and ducts. (See demonstration no. D46.)

- (173) 129. *The relation of the hypophysis to growth of the mammary glands in the rat.* Warren O. Nelson, Wayne University, College of Medicine.

The importance of the hypophyseal lactogenic hormone in lactation cannot be questioned. However, disagreement exists upon the relation of the pituitary to mammary growth. That the pituitary does cause mammary development was first suggested by Corner ('30) and supported by Nelson and Pfiffner ('31) and Lyons ('33). Turner and his co-workers until recently held that only the ovarian hormones stimulate mammary growth.

Work on hypophysectomized animals has shown variable results, but in the rat there is little doubt that the ovarian hormones can develop the glands only in the presence of the hypophysis. Nelson and Tobin ('36) showed that lactogenic plus ovarian hormones was no more effective than only the latter in hypophysectomized rats, but oestrin plus an unfractionated pituitary extract was effective in developing the ducts and alveoli.

These experiments have been extended and confirmed. In an attempt to determine the hypophyseal factor involved in mammary development a series of fractions have been employed alone and in combination with oestrin. None of these was completely effective. Likewise cortin and desiccated thyroid alone or in combination with oestrin have failed.

Despite the present disappointing results there seems to be neither necessity nor reason for claiming that a hitherto unrecognized hypophyseal hormone is demonstrated. Possibly some untried combination of hypophyseal hormones may be responsible for mammary development.

- (142) 130. *The mutual compatibility of embryonic fish and amphibian tissues.* Jane M. Oppenheimer, University of Rochester and Yale University. (Introduced by J. S. Nicholas.)

The compatibility of developing fish and amphibian tissues has been tested by implanting parts of teleostean embryos (*Epiplatys fasciolatus*) either into the blastocoele of *Amblystoma punctatum* gastrulae or into the head or tail bud of *Amblystoma punctatum* and *Triturus torosus* embryos of tail bud stages. The grafts were parts of fish blastulae or gastrulae and parts of premitile and motile embryos: eye, ear, brain, heart and portions of the tail or trunk containing spinal cord, notochord, gut and somites. The hosts were allowed to develop for varying lengths of time up to 2 weeks.

Grafts have been recovered from 61% of the 127 hosts studied in section. All the types of tissue tested are capable of development and differentiation in the amphibian environment. The presence of the fish grafts has no detrimental effect on the adjacent host tissues. The mutual compatibility of the fish and amphibian tissues is conclusively shown since the epidermis and cartilage formed by fish cells is sometimes morphologically continuous with that formed by amphibian cells. The fish grafts are occasionally innervated by nerves originating from the amphibian cranial ganglia. It is concluded that there is no specific incompatibility between fish and amphibian tissues at the stages involved in these experiments.

- (172) 131. *The effects of gonadotropic and estrogenic hormones on the ovaries of hereditary dwarf mice.* Clinton M. Osborn, Harvard University. (Introduced by Frederick L. Hisaw.)

Dwarf mice were obtained from outcrosses from the original strain of black silver mice in which Snell first described the mutation. Their ovaries contain follicles at least three-quarters as large as those in normal mice and show well formed antra and cumuli oophori. After reaching maximal size, the follicles become atretic. Ovulation and formation of corpora lutea do not occur.

F.S.H. injected into dwarf mice over 50 days old caused an increase in number and size of antra-containing follicles. Injections of a highly purified F.S.H. preparation produced small corpora lutea and hemorrhagic follicles although the same preparation failed to produce corpora in sexually immature rats. Whether luteinization was due to traces of L.H. in the F.S.H. or to L.H. produced by the dwarf's own A.P. cannot be shown definitely in this way. The large follicles in dwarf ovaries are very sensitive to L.H. and can be luteinized without preliminary treatment with F.S.H.

Injection of three R.U. of P.U. (total dose) over 4 days invariably produces hemorrhagic follicles and luteinization.

Daily injections of six to eight R.U. of oestrone or oestradiol over periods ranging from 6 to 14 days failed to produce luteinization as a result of release of L.H. from the animal's own pituitary.

These experiments are interpreted as indicating that the dwarf A.P. produces a moderate amount of F.S.H. but little, if any, L.H.

- (132) 132. *The reaction of the 48-hour chick embryo to colchicine.* George H. Paff, Department of Anatomy, Long Island College of Medicine.

Forty-eight-hour chick embryos were treated with colchicine alkaloid at various concentrations for varying lengths of time. Depending upon the amount of the drug and the duration of its action retardation of growth and deformities may result. Under optimum conditions areas of rapid growth are delimited by accumulation of mitotic figures. With embryos under such conditions the most constant effects are obtained in the developing lens, the visceral grooves and extreme caudal end of the neural tube. The typical colchicine figure appears as a dense, more or less spherical mass of hyperchromatic chromatin.

- (65) 133. *The development of the vascular pattern of the human lung.* Dwight M. Palmer, Department of Anatomy, Ohio State University.

Two stages may be distinguished in the development of the vascular pattern of the human lung.

The first stage is one of growth and resembles the morphogenesis of the blood supply of other organs. The early vascular channels are a part of the mesenchymal capillary system. The growth of these vessels and the appearance of arteries and veins continues to about the nineteenth week of development. During this period the stroma of the lung is rather avascular and the relatively few capillaries that are present only occasionally come into intimate relation with the basement membrane of the branches of the tracheo-bronchial tree.

The second stage is one of differentiation or specialization plus growth. This stage begins late in the fifth month of development and is marked by the expansion of capillaries as they come to lie in close relation with the walls of the three or four generations of tubules that lie just orad to the terminal blind end-pieces. In these locations the capillaries give rise to loops that push into the lumina. At first the capillaries are few and far apart in the wall of the tubule. As development continues in the sixth month new vascular buds appear and form more of a meshwork of vessels in the walls of those tubules which become the respiratory portion of the lung.

- (87) 134. *The production of general muscular hypertrophy by the androgenic hormone.* George N. Papanicolaou and Emil A. Falk, Departments of Anatomy and Medicine, Cornell University Medical College, New York City.

Adult male guinea pigs have large and rounded temporal muscles, contrasting with the small and flat temporal muscles of mature females. The difference in these muscles is characteristic of the better development of the muscular system in the adult male.

Female guinea pigs, injected with gonadotropic hormone (follutein) for several weeks, show general muscular hypertrophy. The temporal muscles enlarge and become rounded as in the adult male. The ovaries of such treated females reveal marked hypertrophy of the interstitial tissue.

Male guinea pigs castrated before maturity have under-developed muscles, with their temporal muscles remaining small and flat as in the adult females. Such castrated males treated with androgenic hormone (testosterone propionate) show general muscular enlargement. Their temporal muscles become enlarged, as in the normal adult male. Similar muscular enlargement can be induced in female guinea pigs, normal or spayed, with androgenic treatment. Estrogenic hormone or progesterone failed to induce such hypertrophy.

In the light of these observations, the higher muscular development of male mammals as compared with females, may be attributed to the androgenic secretion of the interstitial tissue of the testis. The muscular hypertrophy following gonadotropic treatment in normal females is probably due to the stimulation of the ovarian interstitial tissue. The enlargement of the temporal muscles can be recognized by palpation, and used as a criterion for the effectiveness of the treatment.

- (99) 135. *Unclosed foramen ovale from the functional standpoint.* Bradley M. Patten, Department of Anatomy, University of Michigan Medical School.

Various types of 'open foramen ovale' are illustrated and discussed from the standpoint of their functional significance. In this connection the critical factors are the competence of the valvula foraminis ovalis and the relative pressures in the two atria. Three general categories of cases may be recognized on the basis of the functional picture presented. 1) Fortuitous failure of complete fibrous union of a competent valve to a normal septum. This condition of 'probe patency' occurs in 20 to 25% of all adults and has no functional significance in an otherwise normal heart. 2) Induced failure of a competent valve to

fuse with a normal septum. This condition, the significance of which has not been generally recognized, is a sequel to some disturbance elsewhere in the circulation (usually pulmonary stenosis) which prevents the normal postnatal balancing of interatrial pressures thus causing a persistent transatrial flow which in turn prevents fusion of the valvula to the septum. 3) Frankly unguarded foramen ovale. In these cases, because of a defective valvula or a defective septum, transatrial flow may take place either from left to right or from right to left. The volume and direction of the transatrial flow is the result of the relative intra-cardiac pressures existing in the case in question. Defects of this type cause characteristic and clinically recognizable alterations in cardiac proportions, but when uncomplicated by intercurrent disturbances are not incompatible with a long and active life.

- (160) 136. *Some effects of testes transplants in female mice.* Carroll A. Pfeiffer, Department of Anatomy, Yale University School of Medicine. (Introduced by Edgar Allen.)

Females from five inbred strains of mice received testes, in the neck, from litter mate males at birth. The testes took in the majority of cases and the development at autopsy varied from practically complete degeneration to a stage comparable to the testis of a cryptorchid male. The first effect on the host was the masculinization of the clitoris. Vaginal smears were predominantly of the estrous type, not the characteristic 'constant estrous' type found in similarly treated rats.

The ovaries were quite atypical especially in the later stages of the experiment, when they often approximate the senile condition, yet there was evidence of high estrogen secretion. There was usually a great increase in the reti-ovarii portion, the luteal-like and brown pigment cells characteristic of ovaries of estrin treated animals and ovarian grafts in male mice. There was also possibly an increase in interstitial cells. Only a few typical corpora lutea were present. However, ovulation occurred and normal ova were found in the oviducts of animals with the above atypical ovarian condition.

The uterus in the early stages was either quite normal or showed some endometrial hyperplasia. In later stages the endometrial epithelium showed signs of pyometra or more often there was a cystic hyperplastic endometrium equal to that produced after long treatment with 250 i.u. of estrin. The uterine glands invaded the myometrium so that in some cases they came to lie just under the serosa of the uterus.

- (84) 137. *The effect of sympathectomy upon the sex functions of female guinea pigs.* R. A. Phillips and G. N. Papanicolaou, Departments of Physiology and Anatomy, Cornell University Medical College, New York City.

The right and left lumbar sympathetic chains were excised in five female guinea pigs. The oestrous periodicity was followed with vaginal smears, without interruption, for over 12 months. The duration and the various phases of the oestrous cycle showed no significant variation from the controls.

Three of the animals were mated. They were all normally receptive and conceived promptly. Gestation, opening of the pubic symphysis, parturition, and lactation were normal. There were no abortions nor still-births. The intra-uterine and post-natal growth of the young was normal.

A second series of eighteen female guinea pigs has been studied for over 3 months following: a) lumbar sympathectomy, six animals; b) excision of the inferior mesenteric ganglion, six animals; c) lumbar sympathectomy plus excision of the inferior mesenteric ganglion, six animals. The length of the oestrous cycles and the duration and character of the various oestrous phases appear to be within the normal limits, except for a slight prolongation of the intervals in group C.

(133) 138. *Autoplastic interchange of visceral arches in Amblystoma punctatum.*

Jean Piatt, Department of Anatomy, University of Vermont. (Introduced by Walter A. Stultz.)

Autoplastic interchange of the hyoid arch and first branchial arch was done on embryos of *Amblystoma punctatum*. All operations were performed at Harrison's stage 32 and on one side only. The ectoderm, mesectoderm, mesoderm, and endoderm of each arch were included in the graft. The experiments were designed to test any specificity relationship which might exist between the seventh and ninth cranial nerves and their respective muscle anlagen. At stage 32 the hyoid arch lies immediately below the geniculate ganglion and the first branchial arch below the visceral ganglion of the glossopharyngeus. The operation effected a reversal of this relationship at a time prior to nerve outgrowth, but the ectopic arches are still close enough to their original nerve supply so as not to preclude the possibility of a normal innervation. Serial sections of twenty-one animals demonstrates a) the almost total absence of muscles derived from either arch, b) the complete suppression of the glossopharyngeal nerve, c) the innervation of all hyoid arch muscles (when present) by the ramus jugularis of the seventh nerve, d) the complete innervation of the first branchial arch muscles (when present) by the ramus jugularis. The results of the experiments indicate a lack of specificity between outgrowing cranial nerves and their respective visceral musculature.

(176) 139. *Studies on the endocrines of reptiles. I. The morphology of the pituitary gland of the lizard (Anolis carolinensis) with special reference to certain cell types.* Ethel G. Poris, Washington Square College, New York University. (Introduced by Robert Chambers.)

The anatomy of the pituitary in *Anolis* is characteristic of reptiles. The stalk is directed posteriorly. The gland lies in the basisphenoidal cavity and is completely surrounded by the bone posteriorly. The pars anterior extends beyond the basisphenoid above the presphenoid cartilage. The gland consists of pars anterior, intermedia and nervosa with no evidence of a tuberalis. The pars anterior is most ventral and is attached to the intermedia only posteriorly. The intermedia which appears to be the largest portion of the gland completely surrounds the nervosa except at the anterior end. Numerous blood vessels penetrate the gland, especially its anterior lobe.

Histologically, the pars anterior shows differentiation between its anterior and posterior portions. The anterior part contains columnar cells filled with intensely eosinophilic staining granules and bordering the blood sinuses. Basophiles and chromophobes are also present. The posterior part contains larger sinuses, bordering on and between which occur ovoid cells containing a vesicular nucleus in a homogeneous non-granular cytoplasm staining lightly eosinophilic with Masson's. Ventral to these is a basophilic region. The intermedia appears filled with columnar cells arranged in rows and bordering on fine lumina. These cells have basal nuclei and a finely granular cytoplasm staining red-purple. Cystic accumulations of amorphous, peripherally granular material, and large oval cells containing a homogeneous reddish-orange non-granular cytoplasm, occur between the bases of the columnar cells.

- (175) 140. *The morphogenesis and cytological differentiation of the chick pituitary.* Hermann Rahn, Department of Zoology, University of Rochester. (Introduced by B. H. Willier.)

The chicken pituitary reveals a bilobed structure of the pars anterior which becomes established at the sixth day of incubation. These lobes which are anterior and posterior are termed respectively 'cephalic' and 'caudal.' The development of the pars tuberalis, the fate of the hypophysial stalk, and the disappearance of the residual lumen are traced. The infundibular process throughout development is separated from the pars anterior and its innumerable diverticula communicate with the third ventricle.

A modified Severinghaus technique reveals at about 8 days a slight basophilic tendency of the pars anterior cells. At 10 days the first unmistakable signs of acidophils arise at the most anterior end of the 'cephalic' lobe and during the succeeding days spread posteriorly producing by the eighteenth day a strong acidophilia in both lobes. Definite basophils, although few in number, appear about the same time. Both are found in the pars tuberalis with the chromophobes. After 18 days permanent changes divide the pars anterior in two cytologically distinct lobes. The 'caudal' lobe represents typical pars anterior tissue; the 'cephalic' lobe is chiefly characterized by an exceedingly faint-staining acidophil of different color and finer granulation. All cells are relatively smaller and more crowded. Morphologically there is no evidence for a pars intermedia, though in a corresponding region the cytology reveals a few cell cords which can be variously interpreted.

- (166) 141. *The nerve fibers of the human hypophysis.* A. T. Rasmussen, Department of Anatomy, University of Minnesota.

The human infundibulum may contain over 50,000 nerve fibers that descend into processus infundibuli. While some pass down among the cell columns of pars infundibularis (narrow sense), it is not clear that they terminate on gland cells. Fibers may invade pars intermedia, but large stretches of this lobe may contain no demonstrable nerve fibers. Those in pars intermedia may be only passing through the epithelium or in most cases the epithelial cells have invaded the neural lobe.

A few fibers from the infundibulum stray into the stroma of pars distalis. Occasionally a few fibers from processus infundibuli pass through pars intermedia to pars distalis where the residual lumen has been obliterated and at the margin where anterior and posterior lobes are continuous.

Nerve fibers from the cavernous plexus enter the hypophysis mostly with blood vessels. Two prominent groups enter the top of the gland, one on each side of the stalk. These penetrate pars distalis deeply, following along prominent connective tissue trabeculae. Individual fibers stray into the epithelial masses. Fifty fibers may accompany a single artery in this region. Small bundles spread out in the capsule mostly of the anterior lobe and at intervals pass into pars distalis usually in the neighborhood of veins or arteries. These fibers are soon disseminated and rarely penetrate very far into the gland. (See demonstration no. D52.)

- (121) 142. *The origin and distribution of the so-called olivary peduncle.* G. L. Rasmussen, Department of Anatomy, University of Minnesota and Department of Anatomy, Medical College of the State of South Carolina. (Introduced by A. M. Lassek.)

Certain points regarding the distribution of fibers arising from the superior olive of the cat and opossum were determined with the aid of various neurological methods. Small lesions were placed in the different portions of the olivary complex and the material treated by the Marchi method. Nissl, Golgi and Bielschowsky preparations served to supplement certain deficiencies of the Marchi method.

Lesions involving the medial three segments of the olive produce degeneration of the so-called olivary peduncle. The distribution of the peduncle is far more extensive than commonly recognized. Only a minor part of its fibers terminate in the abducens nuclei while the remainder disperse through the reticular formation to terminate in various motor nuclei. Most fibers cross in the dorsal half of the reticular formation and then diverge in ascending, descending and transverse directions. The ascending fibers are greater in number and end principally in the dorsal nuclei of the lateral lemniscus. The descending fibers are few in number and terminate chiefly in the inferior olive. The transverse coursing bundle has received a variety of names and interpretations. Our observations indicate this to be visceral efferent fibers which arise, in part at least, from small multipolar cells intimately associated with the medial segments of the olivary complex and course peripherally in the great superficial petrosal nerve. Its ultimate termination is at present undetermined.

- (89) 143. *Uterine changes during gestation and their relation to its end.* Samuel R. M. Reynolds,¹ Department of Anatomy, University of Rochester, School of Medicine and Dentistry.

Published work by a number of authors has established that the uterus grows to accommodate the products of conception for only a restricted period during the course of gestation. Uterine growth begins about coincidentally with nidation and it terminates in the last quarter, fifth or eighth of pregnancy, according to

¹ Fellow, John Simon Guggenheim Foundation.

the species (rat, rabbit, cow). As uterine growth diminishes the products of conception increase in size at their most rapid rate, thus inevitably imposing an increasing degree of tension upon the uterine wall and its contents.

Coincident with this increasing disproportion in rates of growth between the uterus and its contents is a marked reduction in the volume flow of blood circulating through the uterus. The available data show that this takes place simultaneously with the cessation of uterine growth, yet the fetuses and their associated structures increase in size most rapidly at this time. It is therefore clear that as pregnancy nears term the uterine contents tend to exceed the capacity of the uterus to hold them, and at the same time effect a diminution in the source of their own nutritional supply, namely, the circulation of blood through the maternal placenta.

(207) 144. *Similarities and differences between leukemic lymphocytes and tumor cells in mice.* Maurice N. Richter, Department of Pathology, College of Physicians and Surgeons, Columbia University. (Introduced by Hal Downey.)

The following characteristics of leukemia and leukemic lymphocytes in the mouse are similar to the corresponding characteristics of mouse tumors:

1. Leukemic lymphocytes are less mature and proliferate more rapidly than normal lymphocytes.
2. Leukemic cells infiltrate normal tissues.
3. Leukemic lymphocytes originate in definite foci (often multiple foci), rather than by a systemic hyperplasia of lymphoid tissue.
4. The metabolism of leukemic cell populations differs from the normal and is comparable to that of tumors.
5. Leukemia can be transmitted indefinitely through susceptible animals by inoculations of living cells.
6. The interval between inoculation and death decreases during the first several transfers.
7. Genetic factors influence the occurrence of spontaneous leukemia and the susceptibility of animals to inoculation.
8. Susceptible mice can be rendered immune to the inoculation of leukemic cells.
9. Leukemia transmitted by inoculation results from survival and proliferation of cells introduced.
10. Transition from tumor (lymphosarcoma) to leukemia or vice versa occurs spontaneously and has been produced experimentally.

The terms 'leukemia' and 'tumor' are interchangeable in the above statements. The main differences are:

1. Leukemic cells enter the circulation more readily and become more widely disseminated than do tumor cells.
2. Abnormal cells are less common in leukemia than in tumors, and are related to degenerative changes in the tissues.

- (97) 145. *Conduction pathways in the dog's heart.* Robert C. Robb and Jane Sands Robb, Departments of Anatomy and Pharmacology, College of Medicine, Syracuse University. (Introduced by Walter F. Greene.)

By measurement of the times of arrival of the excitation wave in various regions of the ventricle (under several controlled conditions) we are attempting to amplify the details obtainable from gross and histological studies of the Purkinje system.

The apparatus serving this purpose was designed for us by the Cambridge Instrument Company and consists of three crystal torsion galvanometers with balanced push-pull amplifiers, focused on a 12 cm. camera through which photo-sensitized paper may pass at speeds up to nearly 2 meters per second. In some circumstances the parallax and reading errors may each be as small as 1/10,000 second. This apparatus is considered to be the most satisfactory hitherto available for the study of conduction pathways in the heart.

- (158) 146. *The deciduomal reaction in Macacus rhesus.* I. Rossman, The University of Chicago. (Introduced by G. W. Bartelmez.)

Traumatization of the non-pregnant macaque uterus under proper hormonal conditions results in: 1) Proliferation of the surface and superficial glandular epithelium. 2) Endothelial cell hypertrophy and proliferation especially in superficial veins. 3) Swelling and multiplication of fibroblasts with formation of a stratum compactum composed of cells analogous to human decidual cells. A study of the epithelial proliferation from inception to almost complete degeneration at 21 days has been made. Details concerning the Golgi apparatus, centrioles, glycogen, fat, and mitochondria will be presented. The sequence of cytologic events indicates that (in spite of maintenance of the hormonal environment) epithelial proliferative activity ceases because of inadequate circulation. This is due to a passive venous hyperemia, associated with the conversion of superficial veins into large lacunae. During necrosis, much glycogen and fat plus other products of degeneration are released, and may be interpreted as pabulum for a possible embryo. Hemorrhage, with demonstrable rhexis, occurs at this time, and may be apparent externally. This probably contributes to the 'placental sign' bleeding, part of which is thus shown to be independent of trophoblast activity. The effects of the anoxemia on other elements in the mucous membrane will be discussed. The suggestion is made that the hypertrophy and multiplication of decidual cells in areas of epithelial proliferation is a modified form of the fibrosis reaction that occurs in other organs under conditions of prolonged passive hyperemia.

- (4) 147. *Tubercular allergy without infection.* Florence R. Sabin and Austin L. Joyner, The Rockefeller Institute for Medical Research.

For a long time it was thought that the tuberculin reaction could not be induced without infection but subsequently it was shown that it could be induced with heat-killed bacilli. Furthermore, with improved methods of preparation of the protein it was found possible to sensitize with it but only with relatively large amounts.

We have been studying the cellular reactions to fractions from tubercle bacilli and now report that a mixture of tuberculo-protein with tuberculo-phosphatide is a potent sensitizing agent. With this mixture it is possible to sensitize guinea pigs to the protein with an extremely small amount of protein. The addition of the phosphatide seems to have increased the reactivity of the animal to the protein far beyond the activity which can be induced by the same amount of the protein. We believe that this enhancement of allergy is brought about by the cellular reaction to the phosphatide.

- (35) 148. *Excessively large parietal foramina and parietal bone deficiencies.*
J. Parsons Schaeffer, Daniel Baugh Institute of Anatomy, Jefferson Medical College.

In man, greatly enlarged parietal foramina and large osseous deficiencies above the parietal eminences are not identical; moreover, must not be mistaken for certain pathological conditions. Not only are these variants of morphological interest but of importance clinically, especially in interpreting roentgenograms of the living head. Both conditions result in variously-sized soft areas devoid of bone on the vertex of the head. Evidence is at hand which strongly indicates that these abnormally large parietal foramina are both congenital and hereditary. Five generations, in order, show the anomalous condition. In the closure of the parietal or sagittal fontanelle the normal parietal foramina are formed; this being likewise true for the abnormally large parietal foramina. The trait for the formation of excessively large parietal foramina appears definitely inherited. The parietal impressions (depressions), the greatly thinned areas of the parietal bones above the parietal eminences occasionally encountered, are the precursors of large parietal bone deficiencies. The thinned area may be bilaterally present, or a parietal impression may be extremely thinned on one side and a large osseous defect exist in the corresponding field of the opposite side, the thinned area giving way entirely in the formation of the bone deficiency. It is still a moot question whether parietal impressions and deficiencies result from atrophic changes, occurring only in old age, or are congenital and hereditary in character.

- (184) 149. *On dark and light cells in the liver.* Ernst Scharrer, Department of Anatomy, The University of Chicago. (Introduced by C. Judson Herrick.)

More than twenty authors, e.g., Rumjanzev ('27), Böhm ('32), Pfuhl ('32), Clara ('31, '32, '33), etc., describe dark and light cells in the mammalian liver and discuss their functional meaning with complete disagreement regarding their role in production of bile, content of fat and glycogen, behavior toward vital dyes, status as disintegrated or dying cells, etc. Such controversial statements suggest that those cells, whose number varies greatly in different livers, are in reality not intravital constituents of the liver, but artefacts brought about by trauma before fixation when pieces are cut from a fresh liver with pressure on the parenchyma or pulling of connective tissue. Artefacts so produced are well known in nervous tissue. Our experiments show that successful intravascular perfusions of livers of opossums, rats and rabbits with the fixing fluid reduce the number of dark and light cells practically to zero. The same is true in nervous

tissue. The fixed cells cannot react with changes in stainability to pressure exerted directly on the parenchyma or transmitted to deeper parts of the organ through blood vessels and connective tissue. The problem of the dark and light liver cells, therefore, is no longer one of functional role, but rather one of the vehement supravital reaction of parenchymatous cells to mechanical injuries.

- (83) 150. *Changes in the genital tracts of guinea pigs associated with cystic and 'interstitial gland' ovaries.* Ida Genther Schmidt, Department of Anatomy, University of Cincinnati.

Irradiation of the ovaries with a minimum sterilization dose of x-rays leads to 1) replacement of the normal ovarian tissue with 'interstitial gland' and 2) irregular cycles with prolonged oestrous openings, associated with mammary gland development and the urinary excretion of small quantities of theelin. In time, ovarian cysts formed; within a year, endometrial cysts were common. In 2 years, ovarian cysts were huge; uterine glands were numerous, irregular and distorted; the smooth muscle was greatly increased, even replacing the tunica propria. The most severe changes were seen in one animal whose vaginal membrane had been ruptured for 20 months, during which time there was a constant sloughing of cornified and nucleated cells in the vagina. Both uterine horns were enlarged enormously. One horn formed part of a large mass which involved 1) patches of cervical mucous epithelium and stratified squamous cornified epithelium in addition to uterine changes as above, 2) a huge ovarian cyst, 3) cystic expansions of the oviduct and 4) adhesions to the colon, ureter and pancreas. The cervix contained an increased amount of smooth muscle and patches of stratified squamous epithelium, besides its normal mucous epithelium. The vaginal epithelium sent long, anastomosing papillae into its fibrous tunica propria.

Most of these changes are similar to those described in the literature after prolonged injection of oestrogenic substances, or occurring in humans as the result, presumably, of excess theelin in the ovaries.

- (143) 151. *The behavior of transplanted embryonic pancreas in a urodele.* Norman D. Schofield, Department of Anatomy, University of Minnesota. (Introduced by R. F. Blount.)

Factors involved in pancreatic development were studied in *Ambystoma maculatum* employing embryonic transplants, thus avoiding host reactions from exocrine secretion. These animals may be sectioned entire and the graft's relationships observed.

A series of homoplastic transplantations was performed in embryos from stages 36 to 46. The grafts were placed in the peritoneal cavity in the region of the stomach, pylorus or lower gut. The hosts were killed at intervals and mounted serially.

Grafts developed only when the donor was about stage 40 or older. The host must be of a stage to furnish adequate vascularization to the implanted tissue (about stage 40). The grafts show marked differences according to position. The gastrointestinal tract just caudal to the pylorus may exert a positive histotropism, since proliferated ducts invade it. Cranial to this region, although ducts

developed, the relatively thick musculature acted as a barrier to connection. The lower gut does not produce any response in the pancreatic tissue, and, although vascularized, the implants eventually disappear.

Under favorable conditions the transplanted tissue presents the normal appearance of larval pancreas.

Graft maintenance depends largely upon vascularization which enables it to withstand the phagocytic offensive of the host, and the availability of some hollow viscus for the reception of proliferating ducts to give escape for secretions which otherwise would induce autolysis.

- (209) 152. *The growth and calcification patterns of the human deciduous teeth.* Isaac Schour, Department of Histology, College of Dentistry, University of Illinois.

The morphological pattern of the tooth is determined by the dentino-enamel junction outlined by the formative cells prior to appositional growth. The latter begins at definite growth centers (characteristic in number and position for each tooth class) at definite times and proceeds along the dentino-enamel junction at regularly graded intervals. The growth potential is expressed in definite and characteristic rates (average, 4μ per day) and gradients (the rate decreases with age of formative cells, distance from growth center, and from anterior to posterior teeth) of growth, and for definite physiologic life span of formative cells (ameloblasts, 250 days average grading to 0; dentin forming cells, approximately constant, 350 days).

Incremental cones are apposed at each growth center, one above the other in enamel and one within the other in dentin. When the periphery of cones of two adjacent growth centers meet, they fuse and apposition continues along the fused pattern.

The calcification pattern follows the appositional pattern closely. Characteristic and qualitatively different zones and rings superposed upon a basic 16μ incremental rhythm reflect the nutritional adjustments of the child during its development: prenatal zone, very well calcified; neonatal ring; infancy zone, less well calcified; transitional ring (possibly correlated with weaning process); early childhood zone, better calcified than infancy zone.

Deviations from above patterns reflect metabolic disturbances coincident with disease and provide diagnostic features in tooth-ring analysis. (See demonstration no. D55.)

- (149) 153. *The number of vertebrae and the relative length of the spinal regions in primates.* Adolph H. Schultz, Department of Anatomy, Johns Hopkins Medical School.

The lengths of the various regions of the vertebral column were measured from the centers of the intervertebral disks in 300 eviscerated, fresh, catarrhine primates. Man and the anthropoid apes differ from the lower catarrhines not only by possessing fewer thoraco-lumbar and caudal vertebrae and more sacral vertebrae, but also by having comparatively longer cervical, thoracic and sacral regions and much shorter lumbar regions. Chiefly the cervical but also the thoracic

vertebrae are on an average proportionately larger in all higher primates than in the lower catarrhines. Among all primates examined man has the relatively longest cervical and thoracic regions and the comparatively largest lumbar vertebrae. The common evolutionary trend among higher primates to reduce the number of vertebrae and the relative length of the lumbar and caudal regions has gone to greater extremes in some anthropoid apes than in man.

In a series of eighty adult gibbons with much greater vertebral variability than in any human series it can be demonstrated that a reduction in the number of thoraco-lumbar vertebrae is not accompanied by a corresponding reduction in the relative length of this region. A close correlation exists between decreased numbers of thoraco-lumbar vertebrae and increased numbers of sacral vertebrae. It appears that the sacrum increases its number of vertebrae less frequently at the expense of the coccygeal region.

- (194) 154. *The electron microscope*. Gordon H. Scott, Department of Anatomy, Washington University School of Medicine, St. Louis.

In 1926 it was discovered that electrons in motion behave as if they were waves rather than corpuscles. Since then an increasing number of investigations in physics have served to demonstrate this wave-like quality. Among them has been the development of the electron microscope by Brüche, Knoll and Ruska. The light waves of the ordinary visual microscope have been supplanted by electron waves and the glass or quartz lenses replaced by magnetic or electrostatic fields. This instrument has been adapted to use with biological materials. The apparatus will be described; some results obtained by its use will be illustrated and interpretations offered.

- (178) 155. *The effects of androsterone and testosterone on the testes of hypophysectomized guinea pigs*. E. F. Scowen, Department of Anatomy, College of Physicians and Surgeons, Columbia University. (Introduced by P. E. Smith.)

The maintenance of spermatogenesis in hypophysectomized rats by the injection of androgenic substances has been shown by McCullagh et al. and Nelson. The maintenance could not be effected in hypophysectomized monkeys by Smith and Engle, although the dosage which they used was rather small in comparison. It seemed desirable, therefore, to ascertain whether spermatogenesis could be maintained by similar treatment in hypophysectomized guinea pigs.

Adult male guinea pigs were hypophysectomized by the parapharyngeal approach, the completeness of the removal being checked by subsequent serial sections of the pituitary capsule. Injections of either androsterone or testosterone (1.5 mg. and 3 mg. daily) were begun 24 hours after operation and continued daily for 20 days. A unilateral orchidectomy was performed after 10 days and the animals sacrificed and autopsied after 20 days of treatment. Untreated hypophysectomized guinea pigs of the same age and weight were used for comparison under the same experimental conditions. Twelve treated and three untreated hypophysectomized animals were used.

There was some maintenance of testicular weight during the first 10 days of treatment, but during the next 10 days the weight dropped rapidly although it did not reach the low level of the untreated animals. In spite of this weight maintenance there was no evidence of spermatogenetic maintenance in the seminiferous tubules, although the dosage of androgenic substance was sufficient to maintain the accessory reproductive organs at a normal weight.

- (181) 156. *Studies by the freezing-drying method on the nature of the internal reticular apparatus of Golgi and on the granules formed by neutral red in somatic cells of the guinea pig.* William L. Simpson, Department of Anatomy, The University of Chicago. (Introduced by R. R. Bensley.)

Free-hand sections of frozen-dried normal and neutral red injected guinea pig epididymis, spinal ganglion, liver, duodenum, and pancreas have been studied after clearing in paraffin oil and after treatment with various reagents. Results have been correlated with materials prepared by standard cytological methods. 1) The Golgi apparatus may be seen in the usual position in unstained cells of tissues to which no reagent other than paraffin oil has been added. It appears as a thread-like net of slightly different refractive index from the adjoining cytoplasm. 2) After complete extraction of lipoids from frozen-dried epididymis and spinal ganglion by prolonged boiling in absolute alcohol and anhydrous chloroform, the Golgi apparatus still appears in unstained preparations. It may be stained positively after this treatment by Weigert's resorcin-fuchsin. Proof is offered thereby that the Golgi apparatus is not entirely composed of lipoids. 3) The granules formed in cells by neutral red injection are usually found near the Golgi apparatus. Frequently, however, they occupy a position distant from it. 4) The Golgi apparatus persists in recognizable form even in frozen-dried cells where ice crystal artefacts are relatively large. From this and studies of ice crystal formation in other sites in frozen-dried tissues, it may be concluded that the fluidity of the Golgi apparatus is probably less than that of the remaining cytoplasm.

- (195) 157. *A study of the argyrophil fibers of the mouse thymus.* Christianna Smith, Department of Zoology, Mount Holyoke College.

The distribution of argyrophil fibers has been studied in sections of thymus of mice from birth through old age (826 days). From a slight amount at birth, confined mostly to a fine basement membrane outlining the blood vessels, the quantity of reticular fibers increases through puberty and age involution. At 35 days the fibers are distinctly heavier with the veins possessing double or triple sheaths and with more branches found among the cells. From this time on the fibers become more numerous. Especially heavy networks are found among the cells bordering regions which are being replaced by fat. (See demonstration no. D56.)

- (75) 158. *Differences in the time of onset of uterine bleeding after cessation of estrin and of progestin treatments.* Philip E. Smith and Earl T. Engle, Department of Anatomy, College of Physicians and Surgeons, Columbia University.

In spayed and in hypophysectomized-spayed monkeys the period elapsing between the cessation of estrin injections and the beginning of uterine bleeding is different than in progesterone withdrawal experiments. After cessation of estrin injections (100 RU/day for 10 days), uterine bleeding begins in 6 to 17 days whereas after cessation of progesterone injections (pre-treated with estrin), bleeding begins in about 48 hours. After stopping combined estrin-progestin injection bleeding also starts in about 48 hours. These results are in agreement with the periods between ovariectomy and bleeding when the ovaries are removed during the proliferative and during the progravid phase of the cycle. In the proliferative stage, in our animals, bleeding does not take place before 5 days whereas in the progravid stage, bleeding begins in about 2 days. The duration of bleeding is about the same after estrin and after progesterone withdrawal. The short interval after progesterone withdrawal is in harmony with results reported in women after cessation of progesterone treatment, or after corpus luteum removal.

In earlier work in which we injected crude corpus luteum extract a longer period (4 to 8 days) elapsed after cessation of treatment than in this later work with purified or synthetic preparations. The former frequently caused abscesses. It seems probable that the longer interval before bleeding was due to a slower absorption of the crude material.

- (100) 159. *Direct observations of minute structural changes in fibers of cardiac muscle during irritation and injury, and the significance of the intercalated discs of the heart.* Carl Caskey Speidel, School of Anatomy, University of Virginia.

Throughout periods of irritation and injury, cardiac muscle fibers of vertebrates (frog, tadpoles and white rats) exhibit essentially the same varieties of minute structural changes as do skeletal muscle fibers. Mild irritations cause fibrillation, Q-disc granulation, and increased visibility of the Z membranes. Such fibers in the heart are still quite capable of rhythmic contraction. More severe injuries may cause the formation of localized retraction clots of myofibrillar substance (Zenker's degeneration).

A thin retraction clot is often formed from the substance of a single sarcomere, or even a portion of one sarcomere. One variety of injury results in the formation of coarse Z membranes and dissolution of Q discs. In skeletal muscle a very thick clot is sometimes formed including as many as thirty or forty sarcomeres.

In both cardiac and skeletal muscle fibers some thin retraction clots, experimentally induced, are indistinguishable from some intercalated discs of the heart. It is suggested that an intercalated disc originates as a typical retraction clot at a place of functional strain or of injury. Subsequent modification may take place.

Methods of inducing irritation and injury include mechanical distortion and treatment with heat, electricity, alcohol, and salt solutions. Illustrative ciné-photomicrographs have been obtained directly from the living tissues. (See demonstration no. D58.)

- (130) 160. *The potency of prospective body ectoderm in the amphibian gastrula.*
Walter R. Spofford, Department of Anatomy, Cornell University Medical College.
(Introduced by C. V. Morrill.)

Prospective body ectoderm in gastrulating embryos of *Amblystoma punctatum* was transplanted homoplastically to the head region of older hosts. When stage 10 (Harrison) was used as donor, brain parts, sense organs, and other structures were induced in the graft. Transplants from stage 12 donors failed to form such structures under the same conditions.

Grafts from stage 10, which were first explanted into Holtfreter's solution for a period of time during which the donors passed through stage 12 and which were then transplanted to older hosts, formed induced structures as readily as direct transplants.

It is concluded that there is a restriction of potency in the prospective body ectoderm during gastrulation. It is further demonstrated that this restriction does not take place if the ectoderm under consideration is separated from morphogenic influences by explantation.

- (163) 161. *Some correlations between oxygen content of umbilical vein blood and fetal respiratory movements.* A. G. Steele, Anatomical Laboratory, Northwestern University Medical School. (Introduced by W. F. Windle.)

Arterial blood oxygen of resting unanesthetized cats averaged 14.8 vol. per cent and, in late pregnancy, after the cats' carotid and basilar arteries were ligated, 13.6 vol. per cent. Under the latter conditions, temperature and blood pressure were seldom altered and respiration was usually faster than normal.

Intrauterine fetal respirations were frequently seen at term; fetal heart rate was 120 to 130 per minute. Almost simultaneously with incising the uterus to deliver fetuses (placentae in place) sudden darkening of umbilical vein blood occurred. Such fetuses became apneic and their heart rate slowed. The uterus soon readjusted itself and, with subsequent contraction and relaxation, umbilical vein blood often became lighter and darker alternately. When veins were red, fetuses became active and executed rhythmical respiratory movements as fast or faster than in utero; heart rate increased from 50 or less to 190 or more per minute. When they became blue, respirations stopped or became very slow and dyspneic and the heart slowed.

Micro-manometric analyses of umbilical vein blood from twelve fetuses showing active rhythmical respirations revealed 2.86 to 8.56 (average 5.89) vol. per cent oxygen. In seven apneic fetuses, oxygen content was 0.15 to 1.38 (average 0.77) vol. per cent. One active fetus whose oxygen content was 6.56 vol. per cent was allowed to breathe air; 10 minutes later oxygen had increased to 9.72 vol. per cent.

- (6) 162. *Structural disharmony: The genetic and developmental independence of the upper and lower jaws.* Charles R. Stockard, Cornell University Medical College, New York.

Two structures which intimately coordinate in function are usually thought to be linked genetically, and regulatory influences insuring their harmonious development have been postulated. The upper and lower jaws are such related structures and must cooperate in the important final function of securing food by seizing, biting and chewing. It is claimed that the jaws develop in connection with separate portions of one and the same branchial arch and this would suggest not only that the genetic characters of the two jaws are closely linked but further that an harmonious developmental regulation might be expected. In the pure line individual, evolution and natural selection have brought about a structural and functional association between the jaws which largely conceals their mutual independence. However, when pure races having different shapes and types of jaws are crossed, this mutual independence of maxilla and mandible is brought to light. It is surprising to find that the type of each jaw is inherited separately and that developmental regulation is not sufficiently strong to bring about structural harmony between the dissimilar types. The hybrid individual may thus exhibit the upper jaw of one race in association with a completely different type lower jaw from the other race, both developed in the same endocrine environment. Such jaws are often morphologic misfits and physiologic failures.

- (138) 163. *Failure of the iris in Amphibia and fishes to induce lens formation in grafts of regenerating tail blastema.* L. S. Stone and Philip Sapir, Department of Anatomy, Yale University School of Medicine.

Over 180 experiments have been made on adult *Triturus viridescens*, young *Fundulus heteroclitus* and larvae of *Amblystoma tigrinum*, *Rana pipiens* and *Rana clamitans*. After the normal lens was excised segments of regenerating tail blastema, varying in ages from 4 to 18 days, were implanted in the eye chamber near the dorsal pupillary margin of the iris. Specimens were preserved from 1 to 56 days after operation. Many were carefully studied within a few days after operation to determine the ability of the eye to induce lens formation from the graft.

In removing the lens, fragments of lens capsule epithelium and occasionally lenticular fibers are sometimes left behind, especially in the older *Rana* larvae. It can be ascertained with accuracy whether any lens material has been left behind but it is impractical to remove the fragments by probing. Regenerating lenses were found in the urodeles derived only from the dorsal rim of the iris as in the normal manner. Whenever lenses occurred in the other cases it was found that they were formed from fragments of the original lens. Often lens epithelium and graft became fused even to the point of one encapsulating the other. The implants usually developed as masses of loose connective tissue. Occasionally some epidermis and a few muscle cells were found. In not a single case was lens material seen developing from implanted blastema.

- (5) 164. *Origin of the gut endoderm in macaque embryos.* G. L. Streeter, Department of Embryology, Carnegie Institution of Washington, Baltimore.

In the macaque embryo the gut endoderm is abruptly demarcated from the yolk sac endoderm. The two are different in origin and in their functional activities. Also, in their anatomical fate they remain as discrete structures, having little to do with each other. That which is usually spoken of as the yolk sac cavity originates as a cleft between the cuboidal cells that are to form the gut endoderm and the flattened cells that are to form the yolk sac. The cavity is thus originally a dual space which only later becomes subdivided into the cavity of the yolk sac and the cavity of the gut.

- (127) 165. *Hearing loss following closed bite.* Leon H. Strong, Anatomical Laboratory, University of Michigan Medical School.

The anterior tympanic artery is a structure posterior to the mandibular head. It is in an immovable position against the bone as it enters the petro-tympanic fissure. Freely anastomotic with the posterior tympanic artery in the fetus, it supplies the tympanic membrane, ear ossicles and their joints. In the adult the anastomosis is interrupted, leaving the anterior tympanic artery for the supply of these structures. Dentists report progressive deafness following loss of teeth with shortened bite, arrested or relieved by lengthening the bite. Loss of bite would drive the head of the mandible back against the anterior tympanic artery, interrupting its flow of blood, thereby disposing the tympanic membrane and the ossicles to an anemia, hence dysfunction.

- (162) 166. *Androgenic hormone of mouse ovaries not identical with testosterone propionate (Oreton).* M. T. Strong and R. T. Hill, Department of Anatomy, Indiana University School of Medicine, Bloomington.

The ovarian androgen, as evidenced by seminal vesical response in adult castrated male mice with ovaries grafted in their ears, is rendered inactive by thyroid feeding. Androgen secreted by the testes of control males is not so inactivated by thyroid feeding (Hill). If any of the available artificial androgens are identical with the ovarian androgen (s) then one might expect that they also would be inactivated by thyroid feeding.

A preliminary assay of testosterone propionate (Oreton) on castrated male mice of the strain used above, showed that injections of 0.125 mg. in 0.05 cc. of oil, on alternate days for a total of three injections, gave an average combined seminal vesicle and anterior prostate weight of 43 mg.; animals receiving five similar injections had accessories of 120 mg. while those receiving ten injections produced accessories averaging 251 mg.

Castrated male mice were given daily feedings (35 days) of a non-toxic amount of desiccated thyroid. Thyroid feeding was then stopped and the animals given ten injections, on alternate days, of 0.125 mg. testosterone propionate. Upon killing the weight of accessories was found to average 322 mg. The fact that the weight of accessories was somewhat higher than that determined in the preliminary assays is suggestive of a possible augmentation of the hormone action by thyroid feeding. Furthermore, the fact that the injected hormone was not inactivated by thyroid feeding, indicates a lack of identity with the ovarian androgen (s).

- (123) 167. *Further observations on the F. solitarius and its nucleus in the human brain.* R. M. Strong, Department of Anatomy, Loyola University School of Medicine.

Additional material, primarily embryologic, has been studied, and a more complete description of the F. solitarius and its nucleus will be made with the aid of lantern slides. This will include fascicles of fiber belonging to the facial, glossopharyngeal and vagus nerves. The probable course of the F. solitarius in the region of the medulla, caudal to the obex, will also be indicated. This was not possible in the paper presented by the author at a previous meeting.

- (135) 168. *The transposition of fore and hind limbs in Amblystoma.* Walter A. Stultz, Department of Anatomy, University of Vermont, College of Medicine.

Previous studies have shown that the hind limb develops disharmonically when the anteroposterior axis of the rudiment is reversed in grafting to an abnormal (heterotopic) position on the body of the host. When grafted to the normal (orthotopic) position the limb always develops harmonically irrespective of the orientation imposed upon it at transplantation. The conclusion is that some factor resident in the normal hind limb region is able to overcome the axis determination existing in the grafted rudiment.

In contrast, other workers have shown that the anteroposterior axis of the fore limb maintains itself after reversal whether the rudiment is grafted heterotopically or orthotopically.

The transposition of fore and hind limbs (heteronomic transplantations) was carried out to ascertain whether the hind limb region would be able to reverse the anteroposterior axis of the fore limb rudiment and vice versa. The results show that such is not the case. Both fore and hind limbs develop disharmonically when the anteroposterior axes of the rudiments are reversed in grafting to the contrasting limb positions. A comparison of the two limbs, however, regarding resorptions and reduplications and the production of inverted, defective and chimaeric limbs reveals certain fundamental differences between the rudiments at the time of transplantation.

- (136) 169. *Reversal of prospective polarity in the dorsoventral limb axis of Amblystoma punctatum (Linn.).* F. H. Swett, Department of Anatomy, Duke University School of Medicine.

Left limb rudiments from embryos in early tail bud stages were grafted to the opposite flank in inverted orientation. From that locus they were removed to a position on the lateral surface of the myotomes. This second operation was carried out upon a series of groups when the original donors of the grafts had attained stages 29, 30, 31, 32, 33, 34 and 35, respectively. The imposed orientation was again dorsoventral. The problem was to find out how early, after transplantation, reversal of the prospective polarity of the dorsoventral axis was accomplished. The myotome site received the graft at the second operation because the results of earlier experiments had indicated the absence of polarity reversing factors in this region of the body. As a result of the procedure outlined, inverted right limbs were produced over an adequate range of stages to permit the conclusion that the dorsoventral axis may undergo a reversal of its prospective polarity somewhat earlier than it normally becomes irreversibly polarized.

These limbs, however, did not develop in exactly the same manner as did original right limbs transplanted to the myotome site—thus indicating that the transformation of a left rudiment into a right was incomplete.

- (7) 170. *Volume and cortico-medullary ratio of the adult human suprarenal gland.* C. A. Swinyard, Department of Anatomy, Medical College of the State of South Carolina. (Introduced by A. M. Lassek.)

At the present time ninety suprarenals have been obtained from both sexes of White and Negro individuals varying in age from 4 lunar months to 82 years of age. All glands were accurately weighed and the volume was measured by water displacement. Serial sections of twelve glands cut at 20μ have been projected and relative volumes determined by use of a planimeter. The planimetric volumes have been augmented to correspond to the true volumes. The paper weight method was compared with the planimeter method and the difference found to be negligible.

The average total volume of each suprarenal was found to be 26.4% larger in the White than in the Negro race. The white female glands averaged 22.8% larger than those of the white male. The negro female glands averaged 11.1% larger than those of the male negro.

The cortico-medullary ratio varied widely; in the white person it averaged 16.2—1 and in the negro 8.4—1. The average volume of the medulla in the white individual was 29.9% less than that of the negro. The average volume of the cortex was 30.7% greater in the white than in the negro. In the small number of glands studied the larger volume of the suprarenal in the white person is apparently due to the relative and absolute greater volume of the cortex.

- (49) 171. *Has the sensation of pressure its own peripheral anatomical mechanism?* I. Maclaren Thompson and H. Stewart Kimball, Departments of Anatomy, Universities of California and Manitoba.

Several years ago it was observed that when a nerve supplying the skin is stimulated by a suitable alternating current, sensibility in the corresponding cutaneous area is differentially dulled, the threshold for pressure being elevated much higher than those for touch, pain and temperature. Subsequent experimentation showed that this reduction of sensibility is a central phenomenon termed masking.

We have further studied touch and pressure by what we call reversed masking. The cutaneous area being under vigorous tactual or pressural stimulation, the threshold for electrical stimulation of the nerve (sufficient to arouse a barely perceptible fluttering sensation) was measured. Tactual stimulation of the cutaneous area significantly raised the threshold for evoking the fluttering sensation (reversed masking), whereas peripheral pressure had no such effect. Hence pressure, which is much more susceptible to masking than is touch, differs from touch also in failing to effect reversed masking.

For reasons that cannot be discussed in the space here available, we regard these experiments as supporting the opinion that the awareness of pressure is not a primary sensation, with special nerve fibers, but is a mental concept integrated from the sensations aroused by impulses conducted along fibers that are not specific in this respect, and hence should not be called pressure fibers, in the sense that we speak of touch, pain, and temperature fibers.

- (32) *172. Studies in the comparative anatomy of the extrahepatic biliary tract.* Stewart C. Thomson, Department of Anatomy, Loyola University School of Medicine. (Introduced by T. T. Job.)

Dissections were made of fifty species of Mammalia representing the orders Marsupialia (6 species—8 specimens), Insectivora (3-4), Chiroptera (12-15), Primates (5-18), Carnivora (7-11), Edentata (1-2), Artiodactyla (3-3), Sirenia (1-1), Hyracoidea (1-1), Perissodactyla (1-2), Cetacea (1-1), Rodentia (9-17).

Gall bladder absent in whale, hyrax, pocket-gopher, deer, zebra, horse, brown rat, rice-rat; present in tree-kangaroo, wallaby, flying phalanger, Tasmanian devil, opossums (two species), moles (two species), short-tailed shrew, bats (twelve species), orange-utan, chimpanzee, macaque, marmoset, red Uakari, bear, binturong, otter, little panda, cat, dog, mink, armadillo, sheep, cow, manatee, porcupine, guinea pig, striped ground squirrel, rabbit, mouse (two species).

Shape, character of wall, and relationship of gall bladder to liver decidedly variable. Study of relationships of pancreatic and common bile ducts shows that: 1) both ducts may enter duodenum separately (porcupine, manatee, pig); 2) ducts may course together to duodenal wall (marmoset, wallaby, hyrax, tree-kangaroo, little panda); 3) pancreatic duct may empty into bile duct at distance from opening of latter into duodenum (binturong, rat, pocket gopher). Distance of point of entrance of ducts from pylorus, and presence of duodenal papilla variable. Papilla prominent (orang-utan, manatee); of medium size (tree-kangaroo, wallaby); or absent (hyrax, binturong). Complete data shown in tabulations.

- (16) *173. Effects of adrenal destruction on the developing rat embryo.* Charles E. Tobin, Yale University. (Introduced by W. R. Coe.)

The adrenals may be partially or totally destroyed in the rat embryo of 17 days and older by intrauterine cauterization. These embryos, after total or partial destruction of the adrenals, live through the remainder of the gestation period. Their weights at autopsy, with few exceptions, were normal for the age of the embryos.

Hyperemia was found in the remaining adrenal tissue of most of the cauterized embryos. This hyperemia appears to be due to an increased blood supply to the remaining adrenal tissue and to a partial circulatory stasis produced by a substance liberated from the cauterized tissues. Other than the hyperemia found in the adrenal tissue, there was no evidence of increased vascularization or circulatory stasis in any of the other tissues of these embryos.

The pituitary, thyroid, parathyroids, and thymus of embryos with total or partial adrenal destruction, show morphological changes suggestive of the changes found in these glands in the post-natal adrenalectomized rat. The growth and differentiation of the gonads and other reproductive tissues which had differentiated before or after the time of cauterization were not inhibited by total or partial adrenal destruction in these embryos.

- (46) 174. *Changes in the semilunar ganglion referable to senescence in man.*
R. C. Truex, Department of Anatomy, University of Minnesota. (Introduced by A. T. Rasmussen.)

Morphological variations in the elements of the semilunar ganglion form the basis of numerous classifications of atypical and pathological cells. Through the employment of the reduced silver method of Cajal and a cresyl-violet Nissl stain we find changes in the nucleus, cytoplasm, cell processes and capsular cells concomitant with age. The incidence and constancy of these alterations indicate they are a phase in the normal physiological evolution of the cells.

Most commonly the neurons shows a vacuolo-granular metamorphosis which begins with the appearance of vacuoles. These vacuoles coalesce and cause a marginization of the anochromatic nucleus. The end result of this process is the formation of a residual nodule at the site of the senile cell body.

Somewhat less frequently the hypothymic neuron proliferates abortive anisomeric processes which often terminate in hypertrophic end knobs. Many of these dendroids remain subcapsular but others go to form profuse pericellular plexuses around neighboring cells. Both of these processes in the neuron are accompanied by a proliferation of its capsular cells.

The presence of pigment granules, corpora amylacia and intimal thickening in the small arteries in the ganglia suggest a hypophyixial basis for the cellular changes. (See demonstration no. D60.)

- (161) 175. *Intra-ocular homotransplantation of prepuberal testes in the rat.*
C. Donnell Turner, Department of Zoology, Northwestern University. (Introduced by C. L. Turner.)

Testes from newborn animals were transplanted bilaterally into the eyes of normal postpuberal males and females and into gonadectomized adults of both sexes. From a total of 335 transplants, 91% have been recovered and serially sectioned or observed subsequent to teasing. The grafts were allowed to remain in the anterior chambers for periods ranging from 10 days to 15 months. In castrated hosts of either sex, some of the transplants produced sufficient testicular hormone to maintain secretory activity in transplanted or intact male accessory organs of reproduction for as long as 15 months. Fully differentiated spermatozoa were identified in fixed preparations and observed in teased supravital material. The capacity of intra-ocular grafts to proliferate spermatozoa was correlated with hypophyseal stimulation and temperature. The temperature of the anterior chamber of the eye was found to approximate that of the scrotum. The structure and function of intra-ocular testicular grafts were found to be conditioned by the kind and amount of gonadotropic hormones secreted by the pituitary glands of the several types of hosts.

- (18) 176. *Variations in structure of the parathyroid glands of dogs.* E. M. Vicari, Department of Anatomy, Cornell University Medical College.

A survey of the parathyroid glands of several hundred dogs of different breeds and their hybrids show breed variations in number, size, location, presence or absence of cysts and structure. Glands vary in volume from 10 to 150 cu.mm.

The variations in structure have been classified into five types: 1) compact, 2) trabecular, 3) mosaic of compact and trabecular, 4) compact with dark patches of dense chromatic nuclei (syncytium), 5) trabecular with dark nuclear patches. These nuclei are usually spherical and dense with chromatin and in some glands they appear pyknotic. Their arrangement is linear or concentric. A gland may have several large nuclear patches each containing from ten to one hundred nuclei, or the gland may contain many small spots each having from two to eight such nuclei. The amount of connective tissue stroma among the nuclear patches varies in different glands. The parathyroid gland types will be discussed with relation to various breeds and hybrid dogs.

(168) 177. *Production of sperm in hypophysectomized ground squirrels (Citellus) injected with substances from urine and blood.* L. J. Wells and M. D. Overholser, Department of Anatomy, University of Missouri.

Fifty-four hypophysectomized males and thirty-two with intact hypophyses were used. Animals were injected and autopsied during months when controls showed normal seasonal aspermia. Hypophysectomized controls killed during period of sperm expectancy showed no spermatozoa. Completeness of hypophysectomy was determined by studying serial sections of the sella, volume of anterior lobe fragments being measured by the planimeter. Presence of spermatozoa was determined by examining fresh epididymis smears and stained testis sections.

Subcutaneous injections were made twice daily for 6 to 42 days. No substance caused sperm formation when injected for less than 19 days.

Prospermin (Squibb; extract of human male urine) induced sperm formation in absence of anterior lobe in five immature males and six adults, following daily administration of 3 to 5 mg. for 19 to 31 days. Six incompletely hypophysectomized males likewise possessed spermatozoa following similar treatment. Daily doses of 2 to 20 mg. for 30 days produced no stimulation of accessories in nine castrates.

Antuitrin-S (Parke-Davis) in daily doses of 50 to 75 R.U. for 30 to 42 days produced spermatozoa in four immature males—three completely and one incompletely hypophysectomized.

Pregnant mare blood serum in daily doses of 25 to 40 R.U. for 29 to 33 days produced spermatozoa in three completely hypophysectomized males (two immature, one adult). Similar treatment induced spermatozoa in five immature incompletely hypophysectomized males.

(140) 178. *Factors controlling feather development of skin grafts made between chick embryos of different breeds.* B. H. Willier and Mary E. Rawles, Department of Zoology, University of Rochester.

Small pieces of skin ectoderm stripped from the dorsolateral head region of 72-hour chick embryos and transplanted to the right wing bud of hosts of the same age but of another breed, results at hatching in large areas of grafted skin covering the entire wing and often adjacent parts of breast, back and thigh. While feather color of the grafted skin is independent of the host, the form of the feathers and their arrangement in tracts are definitely determined by position in the host body. Host graft combinations of S.C. White Leghorn, Rhode Island Red, and Barred Rock breeds have been tested. All combinations gave positive results except those in which White Leghorn was donor.

Since the breeds used represent three different rates of feather growth, acceleration or retardation in the grafted skin by the host can be readily ascertained. It is clearly demonstrated that the rate of growth of the various feather types of the graft is dependent upon the host following exactly its rate. For example, skin from slow-feathering Barred Rocks grown upon fast-feathering White Leghorns, produces wing feathers identical in length with those of the left (control) host wing, greatly exceeding the length of wing feathers of a normal Barred control of the same age. Conversely, the growth of Rhode Island Red wing feathers is slowed down to the Barred Rock rate when the latter is host.

- (64) 179. *Relationship between certain movements of cat fetuses and the fetal oxygen supply.* W. F. Windle, C. L. Bishop and A. G. Steele, Anatomical Laboratory, Northwestern University Medical School.

We have reexamined development of fetal movements with improved methods, taking into consideration maternal respiration, blood pressure, body temperature, oxygen and carbon dioxide blood content. Incising the gravid uterus quickly led to fetal anoxemia. In early stages of anoxemia, fetuses 18 to 30 mm. long manifested simultaneous contraction of large muscle groups when appropriately stimulated and older specimens showed rhythmical integrated activities such as respiration, alternate progression and sometimes intestinal peristalsis. But with blood oxygen high, fetal movements observed in the seconds or minutes immediately after uterine incision were quite different from those just described. Contralateral as well as homolateral reflexes predominated; some could be elicited in specimens as small as 16 mm. not only with mild faradic and pressure stimulation but also by touching hind limbs, forelimbs and various head regions with a single hair even without removing the amnion. Embryos, too small (13 to 15 mm.) to show integrated movements after anoxemia, did exhibit local responses of limbs or head at the moment of exposure. In 18 mm. embryos, tactile (hair) stimuli induced local reflexes when the specimens were well supplied with oxygen but induced strychnin-like contraction of larger groups of muscles as anoxemia built up.

The earliest embryonic movements in the cat are essentially spinal type reflexes. Fetal anoxemia readily depresses these in all specimens and favors 'purposeful' integrated activities in older fetuses.

- (206) 180. *Three types of lymphoid reaction and the differentiating characteristics of the lymphatic elements involved.* B. K. Wiseman and C. A. Doan, Department of Medical and Surgical Research, Ohio State University.

If it be granted that the reproduction of cells is characterized by maturation and division occurring simultaneously, then theoretically, at least, three types of lymphoid hyperplasia are possible. During the past 7 years there have been approximately 325 cases of primary lymphoid disease studied in our clinic, utilizing not only supravital blood and tissue studies but also, more recently when technical conditions permitted, micrometabolic observations on the circulating lymphocytes. Tissue has been derived both from biopsy and autopsy sources.

Analysis of this experience indicates that all primary lymphoid dyscrasias can be divided into the three types referred to above. Lantern slides illustrating these three classes of lymphoid reaction with respect to 1) lymphoid tissue, 2) peripheral blood, 3) morphology of the lymphocytes, and 4) micrometabolism of the lymphocytes will be presented.

(156) 181. *Twinning in marmosets (Oedipomidas geoffroyi)*. George B. Wislocki, Harvard Medical School.

In thirty-two instances of recorded pregnancies or births in marmosets, twins occurred in 85% of the number, one young in the remainder, excepting one recorded case of triplets.

In eight instances the sexes of the twins were as follows: male-male, three cases; female-female, two cases; male-female, three cases. Thus we are dealing with dizygotic twinning.

Placentation is characterized by two placental discs which the twins share equally. Fusion of the blastocysts with complete disappearance of the intervening chorion occurs in presomite stages, so that all evidence of separate origin of the blastocysts is obliterated at a very early period. The circulations of the two organisms become united by anastomoses.

Immediately after ovulation and in the first days of pregnancy, the ovaries reveal two corpora lutea, one in each ovary. Soon, however, the recent corpora lutea become indistinguishable from large masses of luteinized atretic follicles which develop in marmoset ovaries after ovulation has occurred.

In spite of the intimate fusion of the twins, involving anastomotic connections between their circulations, no evidence of 'free-martin' effects has been observed. Sex integrades have not been noticed in postnatal animals. Moreover, examination of the external genitalia and gonads of heterosexual pairs of fetuses shows them to be identical with the respective male or female genitalia of like-sexed pairs.

(190) 182. *The effects of continuous intravenous injection of dextrose on the pancreas, liver and blood sugar level of guinea pigs*. C. Arthur Woerner, Department of Anatomy, The University of Chicago. (Introduced by R. R. Bensley.)

Guinea pigs were subjected to the continuous intravenous injection of from 1 to 3 gm. of dextrose per kilogram of body weight per hour, from 1 to 12 days. Determinations of blood and urine sugar content were made at intervals. Pieces of the pancreas and liver of each animal were fixed promptly and stained for cytological studies.

Changes in 1) the cells of the islands of Langerhans and the acini of the pancreas, 2) the distribution of fat and glycogen in the liver, 3) the blood sugar level, and 4) the excretion of sugar, are correlated to the rate and duration of dextrose injection. (See demonstration no. D64.)

- (95) 183. *Effects induced in pregnant rats by injection of chemically pure carcinogenic agents.* J. M. Wolfe and W. Ray Bryan, Departments of Anatomy and Pathology, Albany Medical College, Union University, and Department of Anatomy, Vanderbilt University Medical School.

Beginning on the first day of pregnancy, thirty-eight rats received daily injections of 5 mg. of 1:2:5:6 dibenzanthracene, dissolved in 0.1 cc. of sesame oil. Injections were continued until autopsy between the tenth and eighteenth days of gestation. Beginning on days 10 to 12 of pregnancy, profuse macroscopic bleeding from the vagina was observed which persisted until the rat was killed. Gross examination of the pregnant uteri of rats killed on days 10 and 11 revealed that implantation and early growth of the fetus was normal. Rats killed later in pregnancy presented striking and progressive abnormalities. Marked hemorrhage was observed in the region of the lateral margin of the placenta, causing distention of the fetal site and of the adjoining uterine lumen. Some sites were hemorrhagic but shrunken. Histologic examination revealed that marked intra-placental hemorrhage and destruction of placental tissue had occurred in the lateral margin of the placenta. The uterine epithelium passing over the decidua reflexa was generally destroyed and large quantities of blood passed into the uterine lumen. Cessation of the growth of the fetus and progressive destruction and resorption of fetal and placental tissue occurred. Contents of the fetal sites usually disappeared by the eighteenth day. Injection of 3:4 benzpyrene and of 1:2 benzanthracene induced a similar pathologic process in the pregnant uteri; injection of the oil medium alone induced no effect.

- (58) 184. *The basis of cortical localization.* Russell T. Woodburne and Elizabeth Caroline Crosby, Department of Anatomy, University of Michigan.

Localization is not an innate property of the cortex as such but a function of the orderly arrangement of the fiber tracts of the nervous system, together with a corresponding specificity in their successively associated nuclear centers. The segmental arrangement of the fibers in the posterior funiculi reflects the specificity of the entering dorsal roots and impresses itself on nucleus gracilis and nucleus cuneatus, the resulting pattern of nuclear localization being projected on the diencephalon through the medial lemniscus. The composition of the lateral spino-thalamic tract and of the ventral secondary ascending trigeminal tract likewise reflects their segmental origin. The distribution of these ascending systems within the ventral nucleus of the dorsal thalamus impresses on this region both a segmental and a functional localization pattern. Thus lower body exteroceptive and proprioceptive impulses enter the lateral caudal portion of the ventral nucleus, and upper body and head impulses its cephalic medial portion, in a segment for segment distribution through the nucleus. The functional pattern is expressed in a dorsoventral distribution. The arrangement of sensory projection fibers preserves this pattern, impressing it on the sensory cortex. The localization pattern of the motor cortex, expressed by the distribution of its efferent projection paths, fundamentally depends on the segmental arrangement of the motor nerves. These observations have been documented by a study of normal and experimental mammalian material.

- (116) 185. *The medial and midline nuclear groups of the thalamus of the rabbit.* M. Wharton Young, Departments of Anatomy, University of Michigan and Howard University.

A study of the medial area of the thalamus of the rabbit reveals many nuclear masses of various size, structure and outline. The nuclei of the midline have been described by substituting the more recent terminology of this region for the numerical designations employed by d'Hollander.

The cell structure of the various nuclear groups and their fiber connections present some interesting phylogenetic relations. The thalamic radiations could be traced to practically all the medial nuclear groups, but very few reached the midline groups. The medial nuclei of the rabbit have fewer cellular commissures interconnecting them than lower rodent forms such as the rat. There are likewise fewer such fiber connections. A study of myelin stains reveals no medullated fibers interconnecting the medial nuclear masses and few such commissures are seen in the silver preparations. The ventral nuclei retain the most distinct commissural connections.

In the rabbit, the rostral and caudal extremes of the dorsal thalamus are completely separated by the third ventricle. Here there can obviously be no true midline nuclei or commissures. Between these extremes, however, the midline is occupied by cell groups variously arranged. This cellular union between the two halves of the dorsal thalamus varies considerably in various mammalian forms. In the rabbit it is relatively less than in the rat, but greater than in man where only the inconstant massa intermedia remains.

- (139) 186. *On the relationship between the acetylcholine esterase content and the development of motility in Amblystoma punctatum (Linn.).* Karl A. Youngstrom, Department of Anatomy, Duke University School of Medicine. (Introduced by F. H. Swett.)

The material was staged physiologically according to Coghill ('29), morphologically after Harrison's unpublished figures, and the acetylcholine esterase content was determined by the pharmacological method with guinea pig ileum (Bernheim and Bernheim, '36). From the morula (stage 8) to late premotile stages (31 to 33) involving about 190 hours (Dempster, '33), there is about a threefold increase in the acetylcholine esterase content. From the time of the first flexure (stage 34) to early swimming stages (38 and 39), representing about 100 hours, there is almost a twentyfold increase in the acetylcholine esterase content. The critical period in the formation of the enzyme obviously coincides with the development of behavior. These data, with what is known of the action of acetylcholine on skeletal muscle, allow a different interpretation of the coil reaction from that suggested by McCouch (Coghill, '29, p. 14).

- (98) 187. *The conduction system in a case of congenital heart block.* Arnold A. Zimmermann and Jack R. Karel, Departments of Anatomy and Pediatrics, College of Medicine, University of Illinois.

The heart of a 7-month-old infant who died of congenital heart disease was studied histologically in view of electro-cardiographic records showing complete heart block (atrio-ventricular ratio 166/55). Gross examination: moderately hypertrophied left ventricle; abnormal right ventricle with a constriction at inferior margin; ductus arteriosus obliterated; small patency of foramen ovale; pars membranacea of interventricular septum absent; a second foramen in muscular septum 1.5 cm. above apex. Seven tissue blocks from pertinent regions were sectioned serially. The SA node is normal. Small ganglia and nerve fibers lie in close proximity of the node. The AV node is abnormal in location and arrangement. Its lower portion is roughly subdivided into three connected parts by the abnormal fibrous trigones. The upper portion of the AV node is approached by Purkinje fibers coursing in the superior margin of the upper interventricular defect. These fibers extend into the supraventricular crest and upper left ventricular wall, but they are interrupted in the fibrotic endocardium covering the right wall of the aorta. There is no true crux commune. From the lowest portion of the AV node slender strands of conducting fibers extend into the endocardium of the left side of the muscular pillar dorsal to the deficient pars membranacea. These strands expand into typical Purkinje fibers supplying the left ventricle. Vacuolar and granular degenerations in disconnected conduction fibers are noted.

DEMONSTRATIONS

D1. Further study of the reaction of certain endocrine glands of immature female mice to treatment with anuran pituitary substance. A. Elizabeth Adams and Barbara Granger, Department of Zoology, Mount Holyoke College.

At the anatomists' meeting in 1937, experiments were reported in which stimulation of thyroids and adrenals of infantile female mice by anterior pituitary substance of *Rana pipiens* had been secured, but no reaction of ovaries, uteri and tubes, and vaginae had occurred (Adams and Tukey, *Anat. Rec.*, vol. 67, Suppl. no. 3, p. 2). It was suggested that several factors might be involved in this negative result, one being the use of too little frog anterior pituitary, although in six cases, ninety-six anterior pituitaries, averaging 104.0 mg., fresh weight, had been administered.

Since that report, much larger doses of frog anterior pituitary substance have been given to female mice, 18 to 19 days old. Litter mates received mouse anterior pituitary, salt solution, or were left untreated. In several cases, after 3 or 4 days of treatment with frog anterior pituitary, the weights of uteri and tubes and vaginae were greater than those in controls injected with salt solution or given no treatment. Furthermore, the histology of these structures in the animals receiving frog pituitary substance gave evidence of a positive reaction to the treatment. Uteri and vaginae in animals given mouse anterior pituitary also reacted positively to this treatment. The response of thyroids and adrenals to the frog pars anterior appeared similar to that secured in the experiments reported in 1937.

D2. Role of the capsule in suprarenal regeneration. Ralph N. Baillif and Dan D. Baker, Department of Anatomy, Louisiana State University Medical Center.

The right suprarenals were removed and the left enucleated in a series of albino rats. Serial sections of the remaining portions of the left glands were studied and regeneration followed from postoperative days 1 to 57. Colchicine was administered to arrest mitosis and thereby locate the most active regions of proliferation. The capsule was found to be an important source for the regenerating cortex, since numerous mitoses were found in its substance as well as in the remaining glomerulosa remnant. The capsule increased in thickness and its inner border became irregular and indistinct due to numerous cells migrating inward to form the new cortex. These processes were most marked on the third, fourth and fifth postoperative days.

Within the regenerating connective tissue of the glands islets of pale-staining cells were encountered which have a morphological resemblance to those of normal medulla. The pericapsular and intracapsular connective tissue of well regenerated glands showed numerous small melanoblasts. (See also abstract no. 10.)

D3. Separation and inversion of the media and intima of the fetal internal carotid.

T. H. Bast, K. P. Knudtson and W. T. Mautz, Department of Anatomy, University of Wisconsin.

In nineteen of ninety human fetal internal carotid arteries studied, the media was separated from the adventitia and was inverted. The inversion is always in the direction of the blood flow, which indicates that the rupture occurred before circulation had stopped. In one set of twins all four internal carotids showed this pathology. Sections and models will be demonstrated.

D4. Demonstration of blood group tests on ancient human bones, with details of technique. P. B. Candela, Brooklyn, N. Y. (Introduced by Thomas Horace Evans.)

The demonstration will consist of the simultaneous testing of two specimens of bone, one of group A, and the other of group B, using the same test sera for both. One of these specimens is of Egyptian origin, and is believed to be at least 3500 years old, and the other was recovered in Minnesota. Although the antiquity of the latter is not well established, its association with the tooth of an extinct species of bison speaks against a very recent date. Its value, however, lies not in its age, but rather in the fact that it demonstrates the feasibility of testing bones recovered from damp, rainy localities, and therefore permits the application of blood group tests to remains from all climates.

The technique will be described with the assistance of a series of diagrams, which illustrate the principles involved, and the errors to be avoided in the performance of blood group tests upon skeletal remains.

The material and the method to be described have been reported in *American Journal of Physical Anthropology*, vol. 21, p. 429 and vol. 23, p. 71.

D5. Microscopic preparations showing structural differences in nerve fibers fixed during activity and inactivity. Eben J. Carey, Department of Anatomy, Marquette University School of Medicine. (See abstract no. 28, and demonstration no. D6.)

D6. Direct observations of the changes in living and intact nerve fibers of the frog by means of reflected light and water immersion lenses. Eben J. Carey, Leo Massopust and Herbert Poetsch, Department of Anatomy, Marquette University School of Medicine. (See abstract no. 28 and demonstration no. D5.)

D7. Photographs and slides of a case of human sexual precocity. J. LeRoy Conel, Department of Anatomy, Boston University School of Medicine, The Children's Hospital, Massachusetts Memorial Hospitals.

Female child 2 years and 2 months old with height of 4 years, weight of 5 years, bone development of $5\frac{1}{2}$ years and sex age of 12 years. Mammary glands began to enlarge at age of $1\frac{1}{2}$ years. Small amount of vaginal bleeding occurred at the age of 1 year and 7 months; no bleeding since then. Pigment about the alveoli and a slight amount of pubic hair appeared at 2 years. Many large cornified epithelial cells present in vaginal smears. Estrone was identified in the urine.

Blood pressure ranged from 102 to 115 systolic and from 60 to 90 diastolic; normal pulse ranged from 80 to 95; normal respiration 20.

Autopsy revealed a tumor involving the entire hypothalamus. Cysts present in both ovaries, especially large in the left; graafian follicles with ripe ova present in each ovary. Many granulosa cells present, but no tumor. No particular pathology observed in any other tissues. Endometrium of uterus is well developed, in pre-ovulatory stage and indicates follicular influence. Vaginal mucosa shows follicular influence, large numbers of flattened cells in the superficial layers.

D8. California Australomorphic crania. Earl W. Count, Department of Anatomy, New York Medical College. (Introduced by C. E. Tharaldsen.)

No. 2503 consistently resembles, in most of the indices taken, the Northern Australians; next to these, the (Baining) Melanesians; to a still lesser, yet appreciable, extent, the 'Pseudo-Australoids' of Hooton.

No. 316 resembles both Australians and Melanesians in these indices; the 'Pseudo-Australoids' to a somewhat lesser extent.

No. 4561 resembles all three referents, but leans slightly to the Australo-Melanesians.

Indices: Cranial, height-length, height-breadth, total facial, upper facial, orbital, nasal, alveolar, gnathic.

No. 2503 and no. 316 are briefly described in the *Zeitschrift für Rassenkunde*, Bd. 7, S. 2 ('38).

They also seem to bear morphologic affinity to some of the Tierra-del-Fuegians.

The position of these Californians may shed light on the mooted questions of comparative morphology and racial affinity of the primitive Oceanians and a portion of the west coast marginal Amerinds.

D9. The blood vessels of the brain substance in some amphibians. E. Horne Craigie, Department of Biology, University of Toronto.

In *Necturus*, *Cryptobranchus*, *Triturus* and *Plethodon* the tissue of the central nervous organs is vascularized entirely by simple capillary loops, the component capillaries of which never branch or anastomose. All other tissues have capillary networks.

In the brains of *Ambystoma tigrinum*, *A. maculatum* and *A. jeffersonianum* both loops and a simple network are present.

In the frog there is a more complicated capillary network with no trace of capillary loops. (See also abstract no. 38.)

D10. Roentgenographs and drawings illustrating the process of ossification in the human laryngeal cartilages. Maxwell Dauer, Departments of Anatomy, College of Dentistry and the Graduate School, New York University. (Introduced by Gustave J. Nobaek.)

Anteroposterior roentgenographs of complete isolated larynges and views of lateral halves clearly reveal the beginning and mode of extension of the ossification centers. These changes are represented by means of drawings illustrating each of the stages in the thyroid, cricoid and arytenoid cartilages. (See also abstract no. 43.)

D11. Protargol staining of paraffin sections of nervous tissue. H. A. Davenport and C. L. Kline, Department of Anatomy, Northwestern University.

The following procedure, based on Bodian's ('36) staining method has given excellent results on tissue of the central nervous system and peripheral nerves in mammals. Fix in a mixture of trichloroacetic acid, 10; formic acid, 5; normal propyl alcohol, 25; and normal butyl alcohol, 60 parts, for about 16 hours. Pass tissue through 95, 80 and 50% ethyl alcohol, about 1 hour each and wash in water for several hours. Dehydrate, embed in paraffin, cut, and mount in usual manner. Remove paraffin and run through graded alcohol to water. Impregnate 3 to 5 days at room temperature with a 0.5% aqueous protargol solution. Reduce 2 minutes with = amidol, 0.5 gm.; sodium sulfite, 5 gm.; water, 100 cc. Rinsing before reduction is optional. Wash, tone with 0.1% gold chloride, and unless the stain is rather dark, wash and reduce with 0.5% amidol without sulfite. If the intensification caused by the second reduction is not desired, treat with hypo after toning. Wash, dehydrate and cover. The stain usually favors the unmyelinated fibers and neurofibrils. Better fixation has been obtained with the 3 and 4 carbon straight chain alcohols in the Hofker type of fixing fluid than with ethyl or methyl alcohol. Acetic acid can be substituted for formic.

D12. The occurrence of two types of acidophile cells in the anterior pituitary of several mammals. Alden B. Dawson, Biological Laboratories, Harvard University.

In the anterior pituitary of the female rabbit and cat (Friedgood and Dawson, '37, and Dawson and Friedgood, '37), after fixation in formalin-corrosive sublimate and staining with Heidenhain's azan modification of Mallory's method, a second type of acidophile cell is intensely and selectively stained with azocarmine, while the ordinary acidophile reacts with orange G. In the unstimulated pituitary of these animals the predominating acidophile is stained orange but during certain phases of the reproductive cycle numerous carmine cells appear.

Somewhat similar cells, reacting less specifically with azocarmine are also present in the pituitary of the female macaque monkey. It has also been possible to recognize two classes of acidophiles in the guinea pig. In this animal the cell reacting with azocarmine is the predominant cell while the cells reacting with orange G are less numerous. Representative slides will be demonstrated. (See also abstract no. 45.)

D13. Frozen tissue fixation. Wilfrid T. Dempster, Department of Anatomy, University of Michigan.

The method consists of combined fixation and dehydration of frozen tissues by immersion in alcohol or alcoholic mixtures maintained at low temperatures by carbon dioxide ice. Advantages and limitations will be pointed out.

D14. Behavior of pigment cells in tissue cultures of the neural crest of the chick. Frances Dorris, Osborn Zoological Laboratory, Yale University. (Introduced by Ross G. Harrison.) (See also demonstration no. D15.)

D15. The production of pigment by chick neural crest in vitro and in grafts to the 3-day limb bud. Frances Dorris, Osborn Zoological Laboratory, Yale University. (Introduced by Ross G. Harrison.)

Neural crest from early somite stages was grown in plasma-extract medium in depression slides for periods ranging from 7 to 15 days. Various breeds were used. Explants from embryos of pigmented breeds, such as the Black Minorca, Australorp and Barred Plymouth Rock, are made up largely of amoeboid cells filled with refractile granules which gradually acquire pigment, reaching full intensity in many cases by the third day in vitro. No pigment cells were obtained from neural crest of Rhode Island Reds or White Plymouth Rocks (recessive white), although cultures of White Leghorn (dominant white, having a color factor plus inhibitor) produced them almost invariably.

When inserted into the limb bud of a 3-day host, neural crest from Australorp (dominant black) produces melanophores in the dermis and in the feather germs of both White Leghorn and White Plymouth Rock hosts, while no pigment resulted from grafts to Rhode Island Red embryos. Neural crest of White Leghorn grafted to White Plymouth Rock produces extensive pigmentation of the dermis, including shanks and feet, and in an extreme case a 14-day host shows a grayish tinge throughout the entire dorsal feather tract, due to the presence of large numbers of pigmented cells in the shafts. (See also demonstration no. D14.)

D16. Synaptic end bulbs in an infant. Donald Duncan, Department of Anatomy, University of Texas.

A demonstration of pericellular end bulbs on the cells of the superior olive which are equivalent in size and shape to those described in relation to the motor cells in adult specimens. In the same individual well-developed end bulbs were found in the nucleus gracilis, nucleus cuneatus and chief sensory nucleus of the fifth nerve. Only occasional small and light staining bulbs were found on the ventral horn cells and motor cells of the fifth nerve. The material was obtained from a white male 3 months old and was prepared by the pyridine silver method.

D17. Pressure distribution in the foot. Herbert Elftman, Department of Zoology, Columbia University. (Introduced by William K. Gregory.)

The motion pictures illustrate a method for studying the correlation between the structure of the foot and the function which it performs by recording the pattern of distribution of downward pressure in the sole of the foot and the changes in this distribution during movement. By this means a record of events in normal and abnormal feet can be obtained and analyzed by slow-motion projection. The records to be shown include normal and flat human feet in various movements and chimpanzee feet in bipedal walking. (See also abstract no. 53.)

D18. Further experiments on the thyrotropic field phenomenon in the tadpole of Rana pipiens. William Etkin, Department of Biology, College of the City of New York and American Museum of Natural History.

It had previously been reported that the experimental approximation of thyroid and pituitary primordia in the tadpole leads to precocious activation of the former and, thereby, precocious metamorphosis in the host tadpole. Further experiments in attempts to elucidate the biological significance of this phenomenon have not yet lead to a consistent interpretation. Some of the facts recently developed will be illustrated by specimens as follows.

1. Histologically, the activation of a thyroid in the thyrotropic field of a tail bud embryo is hardly detectable until after 5 days, reaches a peak at 10 to 15 days and thereafter generally shows loss of activity. The metamorphic effect appears in about 15 days or whenever the host reaches 20 to 25 mm. total length.

2. Grafts of differentiated thyroids dissected from 20 mm. total length donors give essentially the same results as primordia, grafts taken from older donors seem to give less of an effect.

3. Grafts showing activation have been found widely scattered in the dorsum of the head, commonly, for example, in the orbit. Yet if the graft is made directly into the orbit or ventricles of the brain no precocity is induced.

4. Thyroid primordia placed against the infundibulum in 12 mm. total length post embryonic tadpoles showed no precocious activation. In view of item 3, however, this cannot be accepted as critical evidence that the field effect has disappeared at this stage as no cases were found in which the graft actually touched the pituitary.

D19. Human materials from ancient site, Malba, Long Island. Gertrude S. Evans, Freeport, N. Y. (Introduced by Thomas Horace Evans.)

The site yielded twenty-four skeletons, including one child in same grave with adult female. The study deals with the teeth, cranial elements and ear bones. Hair on one of the crania was well enough preserved to permit microscopic section (by Miss Jane Carleton) which will be contrasted with sections and indices from an Egyptian mummy (possible XIXth dynasty). Blood group reactions were taken by Dr. P. B. Candela, on temporal bones. Artifacts accompanying the bones included a nail and a hand-worked stone, possibly of earlier period. Skeletons are North European, possibly of more than 300 years antiquity.

D20. 1. Concomitant variation, at temporomandibular joint, with lines of force suggested. 2. Bony outgrowth of left femur (Egyptian charioteer?) above condyle in adductor magnus. Thomas H. Evans, The Long Island College of Medicine and the New York Medical College.

Two hundred thirty-four crania studied; the changes along Meckel's cartilage are probably progressive. The pterion varies, and metopism is noted. Attempt to distribute by formulae as elements of quanta. The left femur (Egyptian Department, Brooklyn Central Museum) shows effects of occupation, and concomitant structural points, nutrient foramina, third trochanter contribute to explanation. (See also abstract no. 57.)

- D21. The pneumatic spaces in the tip of the petrous bone, an x-ray study.* H. B. Forester, Department of Anatomy, University of Wisconsin. (Introduced by T. H. Bast.)

Reconstruction of serial sections of petrous bones indicate that the air cells of the petrous tip open into the middle ear and not as a rule into the mastoid air spaces.

Centrifugalization of temporal bones whose middle ears were filled with x-ray opaque substances has made possible the visualization of such apical air spaces with the x-ray.

- D22. Parenchymatous cells of the infundibular process and stalk in the rat.* I. Gersh, Department of Anatomy, The Johns Hopkins University.

Sections and photomicrographs will show the normal range of variability in the morphology of the 'glandular' cells of the pars nervosa. In addition, experimental materials will be available that indicate that these cells are involved in the production and secretion of the antidiuretic hormone.

- D23. Motion pictures of living parathyroid and thyroid cells of man and dog.* George O. Gey, Department of Surgery, Johns Hopkins Medical School. (See abstract no. 66.)

- D24. Transitions in the cells of artificially induced mammary tumors in rats and mice.* M. F. Guyer and P. E. Claus, Department of Zoology, University of Wisconsin. (No abstract.)

- D25. Induction of precocious masculine behavior by injection of synthetic androgen.* James B. Hamilton, Department of Anatomy, Yale University School of Medicine.

Stimulation of cock-like behavior occurs after eight daily injections of 0.5 mg. of testosterone propionate¹ in the newly hatched chick. Complex actions such as crowing and strutting are carried out even though the under developed syrinx seemingly does not lend itself to mature polypitch vocalization. (See also abstract no. 73.)

¹ Furnished under the trade name Perandren by Ciba Company.

- D26. Non-effect of superior cervical ganglionectomy on the function of the anterior lobe of the hypophysis.* W. H. Hollinshead, Department of Anatomy, Duke University School of Medicine.

Bilateral superior cervical ganglionectomy was performed on twenty-two rats, twelve males and ten females, during the fourth postnatal week. Eleven male and ten female litter mates of these served as controls. Weekly weighings during the succeeding 8 weeks showed no essential differences between the growth curves of the operated and control animals. The sex cycles of the maturing females, determined by vaginal smears, were normal. Histological examination

of the pituitaries, thyroids, adrenals, and reproductive tracts revealed normal cellular morphology in all cases. The findings indicated that there was no essential disturbance of hypophyseal function following the ganglionectomies. The report of Collin and Hennequin ('36) that an intact cervical sympathetic pathway is essential to the formation of the secretion granules of the basophils and eosinophils has received no support from this work. No functional relationship between the cervical sympathetic and the anterior lobe of the hypophysis was demonstrated by these experiments.

D27. Development of dentine in the teeth of parathyroidectomized rats. Margaret M. Hoskins and Gerrit Bevelander, Department of Anatomy, College of Dentistry, New York University.

Slides and photographs showing the relation of the calcification of dentine to serum calcium, rate of dentine formation, size of the submaxillary, and the general condition of the animal after parathyroidectomy.

D28. Sex reversal in female Amblystoma tigrinum after ectopic implantation of testis preprimordia. R. R. Humphrey, Department of Anatomy, University of Buffalo.

Implantation of gonadal preprimordia into the pronephric region in *Amblystoma* embryos serves to bring ovaries under the influence of a testis throughout their entire development, yet largely or entirely precludes the formation of gonad mosaics through intermixture of gonadal components of grafts and recipient.

In fifty-two *A. tigrinum* (Chicago strain) with such ectopic transplants, the sex type of the gonads at 3 months was determined by exploratory operations to be 11 ♂♂, 15 ♂♀, 14 ♀♂ and 12 ♀♀ (small symbol represents the graft gonad). In the 14 ♀♂ animals, subsequent explorations and final autopsies revealed a development of testicular lobules (tubules) in one or both ovaries of nine (64%) or in a total of fourteen ovaries of a possible twenty-eight (50%). Removal of the graft testis after lobule development had begun in any part of an atrophic ovary was usually followed by hypertrophy of that part and by the appearance of lobules in other regions. In some instances the ovaries thus became transformed into large testes of normal histology; male secondary sex characters attained a corresponding development.

These findings contribute further evidence that the ovary of *Amblystoma*, after inhibition by a testis, may itself undergo reversal to a testis through the growth and differentiation of its medullary and hilar portions.

D29. The origin of sheath cells in the chick. David S. Jones, Department of Anatomy, Loyola University School of Medicine. (Introduced by T. T. Job.) (See abstract no. 92.)

D30. Megaloblasts and normoblasts. Oliver P. Jones, Department of Anatomy, University of Buffalo.

The first demonstration shows a pronormoblast (pro-erythroblast) in a smear of aspirated normal human bone marrow. This cell is closely related to the myeloblast from which it arose and represents the earliest recognizable stage in normal erythropoiesis. Some authors have misinterpreted similar cells for megaloblasts.

The next three demonstrations show various stages of megaloblastic maturation as seen in imprints of pernicious anemia marrow biopsied during relapse. The earliest developmental stage of the pathologic red cell series is the promegaloblast. Note that the nuclear structure of this cell consists of delicate chromatin masses spread uniformly throughout and separated by curved areas of parachromatin (oxychromatin). The fourth demonstration indicates that megaloblasts do not lack the power to differentiate to later stages of development and elaborate hemoglobin.

The last three demonstrations show that cells identical with pernicious anemia megaloblasts are not present in fetal liver and bone marrow from newborn and week-old rabbits. The earliest recognizable stage of normal erythropoiesis in these preparations is comparable to that represented in the first demonstration, namely, the pronormoblast (proerythroblast). (See also abstract no. 93.)

D31. Smooth muscle simulacra of discs of cross striated muscle. H. E. Jordan, Department of Histology and Embryology, University of Virginia. (See abstract no. 95.)

D32. Possible mechanisms for the irregular distribution of chromosomes in androgenetic embryos of Triturus viridescens. Cornelius T. Kaylor, Princeton University and Department of Anatomy, New York University College of Medicine. (Introduced by G. Fankhauser.)

In androgenetic blastulae of *Triturus viridescens*, it has been previously shown (Fankhauser and Kaylor, '35) that some cells may have chromosome numbers below or above the expected haploid number. It was thought that these abnormal chromosome numbers might have resulted from the same types of irregular mitoses which had been seen in the early cleavages of merogonic egg fragments of *Triton palmatus* (Fankhauser, '34, IV and V).

In a preliminary cytological study of a series of seventeen androgenetic blastulae of *Triturus viridescens* which had ceased further development, five blastulae, with delayed and irregular cleavages were found to contain one or more abnormal mitotic figures, such as triasters, tetrasters and possibly pentasters.

These observations show that in androgenetic blastulae of *Triturus viridescens*, produced by the puncturing method of Daleq (Kaylor, '37), multipolar mitoses are the main mechanism by which cells receive an abnormal chromosome complex.

Drawings illustrating the methods which were used to obtain androgenetic embryos of *Triturus viridescens* are shown. Also slides, photographs, and drawings of the irregular mitotic figures mentioned above are presented. (See also abstract no. 58.)

D33. Cellular elements in mouse leukemia. A. Kirschbaum, W. U. Gardner and L. C. Strong, Department of Anatomy, Yale University School of Medicine.

Dry imprints of normal and leukemic hemopoietic tissues, both lymphoid and myeloid, are being demonstrated. Of particular interest is a transplantable myelogenous leukemia which shows all the cytological features of human myelogenous leukemia, a large proportion of the myeloid cells in the blood being immature. These mature to cells which are morphologically identical with the cellular components of normal mouse bone marrow. Auer bodies characterize many myeloblasts just as in some human leukemias. Myeloid metaplasia is found in lymph nodes, spleen and liver. The myeloid stem cell is practically identical with the lymphoid stem cell seen in certain lines of lymphatic leukemia. (See also abstracts nos. 100 and 114, and demonstration no. D39.)

D34. Motion picture of capillary circulation in frog striated muscle, lung, suprarenal, liver and kidney glomeruli. M. H. Knisely, Department of Anatomy, The University of Chicago. (Introduced by R. R. Bensley.) (No abstract.)

D35. The development of plasma cells from reticulo-endothelial cells as seen in successive bone marrow biopsies in the rabbit. Fred Kolouch, Jr., Department of Anatomy, University of Minnesota. (Introduced by Hal Downey.) (See abstract no. 101.)

D36. a. Modifications in autonomic ganglion cells probably related to age.
b. Death of autonomic ganglion cells associated with excessive pigmentation.
 Albert Kuntz, St. Louis University School of Medicine. (No abstract.)

D37. Motion pictures of lymphocytes and monocytes. Warren H. Lewis, Department of Embryology, Carnegie Institution of Washington, Baltimore. (See abstract no. 113.)

D38. The autonomic innervation of the face. F. H. Lewy and R. A. Groff, Departments of Neurophysiology and Neurosurgery, University of Pennsylvania. (Introduced by O. V. Batson.)

The autonomic innervation of the face, as exemplified by the Vulpian-Heidenhain and allied phenomena in in denervated muscles, has been studied in the eyelid, lip, whiskers and tongue. All these phenomena may be elicited by electric or drug stimulation both over the cranial autonomic and the sympathetic nerves. The sympathetic fibers conveying these impulses run in the cervical sympathetics, the corresponding autonomic fibers in the three sensory and in the motor division of the trigeminal nerve, but also in the third, seventh and twelfth cranial nerves. Stimulation of the first trigeminal division elicits a slow elevation of the eyelid, outlasting the stimulus, after degeneration of the oculomotor nerve. Stimulation of the second division leads to a contraction of the canine muscle and to a fan-like spreading of the moustache, after degeneration of the facial nerve. Stimulation of the third division as well as of the chorda tympani produces an arching up of the posterior one-third of the tongue, after degeneration of the twelfth nerve.

Isolated section of one of the above-mentioned trigeminal divisions or of the third, the seventh, the twelfth cranial nerves is followed by a retrograde degeneration in all three portions of the mesencephalic trigeminal nucleus. Direct electric stimulation of this nucleus has the same effect as stimulation of the peripheral divisions of the fifth nerve. The mesencephalic trigeminal nucleus apparently gives rise to fibers conveying the autonomic innervation of the face. Both parasympathetic and sympathetic fibers are effective in producing various phases of the pseudomotor phenomena of the face.

D39. Colchicine effect on normal and pathologic tissues of mice. F. J. Lits, Department of Pathology, University of Brussels and Department of Anatomy, Yale University School of Medicine. (Introduced by Edgar Allen.)

Sections of normal and malignant lymphoid tissues following the administration of colchicine are being demonstrated. (See also abstracts nos. 100 and 114, and demonstration no. D33.)

D40. The effect of excess cholesterol on the Golgi apparatus in nerve and liver cells of the rat. Ross C. MacCardle, Department of Anatomy, Duke University School of Medicine. (Introduced by F. H. Swett.)

This is one of a series of experiments designed to compare the changes in form of cytoplasmic inclusions with functional changes (e.g., fatty degeneration; metabolic disturbances; calcium imbalance). Rats were injected intraperitoneally with 4 cc. of 1% solution of cholesterol in corn oil every 2 days for 30 days.

After such administration of excess cholesterol, the Golgi apparatus of dorsal root ganglion cells (preserved according to methods of Cajal, Helly, DaFano, and Severinghaus' modified Nassonov-Kolatshev) becomes hypertrophied, and then breaks into small globules which become larger by apparent coalescence as they migrate to the peripheral cytoplasm. The Golgi apparatus in normal cells appears black after silver impregnation; whereas in cells treated with cholesterol it appears reddish brown. In liver cells the Golgi apparatus becomes enormously hypertrophied and breaks into components which are indistinguishable from fatty globules. Because of similarity of staining reactions it is difficult to determine whether Golgi material is transformed into fat.

In ganglion cells, after treatment with excess cholesterol, and supravitality stained with neutral red, definite dye-tinted granules occupy a region near the nucleus quite distinct from that of the Golgi apparatus. However, the Golgi material does consist of droplets similar, in size only, to the vacuoles that form around neutral red granules. These neutral red bodies appear in the same locality as do Holmgren's intracellular canaliculi.

D41. Epicytes from the alveolar wall of the cat's lung showing reaction to osmium tetroxide insufflated during life. Charles C. Macklin, University of Western Ontario. (See abstract no. 117.)

D42. Anatomical variations and pathological alterations in the ventricles of the human brain. John Martin, Department of Surgery, Northwestern University Medical School. (Introduced by W. F. Windle.)

Reconstructed ventricles in this demonstration are mouldage casts made directly from autopsy specimens, and the contrast between the normal and pathological ventricles is obvious. Frequently occurring anatomical variations, independent of the attendant pathology, are: the partial or total absence of one or both posterior horns of the lateral ventricles, the frequent fenestration of the tips of the inferior horns, the absence of the intermediate mass, almond sized cyst-like formations in the chorioid plexus of the lateral ventricles, and occasional small out-pouchings of the walls of the lateral ventricles. Pathological variations are due to various types of intracranial disease, such as neoplasms, abscesses, meningeal inflammatory disease, and trauma. These lesions may be cortical, subcortical (thalamic), or intraventricular, and the changes produced fall into definite classifications. Shape and extent of the lateral ventricles are in some cases greatly altered; the third ventricle may be compressed, excavated, or herniated forward, downward, or upward; the cerebral aqueduct may be dilated or compressed, and frequently, with a cerebellar or fourth ventricle lesion, there is a marked internal hydrocephalus.

Such a method of study gives a clear understanding of the bizarre ventricular changes and method of their production in the presence of intracranial disease. It indicates the incidence of several anatomical anomalies which may appear in normal ventricles.

D43. The blood supply of the spleen and its collateral circulation. N. A. Michels, Daniel Baugh Institute of Anatomy, Jefferson Medical College.
Spleens with excised, dissected arteries and drawings. (See abstract no. 125.)

D44. The absorption of thorium dioxide by the reticulo-endothelial system of the dog. Otto A. Mortensen and Russell L. Guest, Department of Anatomy, University of Wisconsin. (See abstract no. 127.)

D45. The wall of bile capillaries in the domestic pig. Thomas H. Morton, Department of Biology, Niagara University. (Introduced by George W. Corner.)

Microscopic slides and photomicrographs exhibiting 1μ segments of bile capillaries are shown with the axes of the capillaries lying in the optical axis of the microscope.

Lines which seem to indicate the free surfaces of the hepatic cells and to originate at terminal bars are seen in the supposed lumina. These lines appear in bile capillaries formed by either two or three cells. There are cases in each type of capillary where a lumen is visible between the lines and more frequent cases where the opposed lines are apparently contiguous.

The region between the lines and that which is generally regarded as the wall of the capillary is acidophil and homogeneous.

D46. *The innervation of the pancreas in the cat.* Russell L. Moseley, Department of Anatomy, University of Minnesota. (Introduced by A. T. Rasmussen.) (See abstract no. 129.)

D47. *The homology of the vesicular ovarian follicles of the mammalian ovary with the coelom.* H. W. Mossman, Department of Anatomy, University of Wisconsin.

Sections of various mammalian ovaries are shown in support of the theory of the homology between the vesicular ovarian follicles and the coelom. These illustrate surface eggs, egg cords or tubes, and everted corpora lutea.

D48. *Preganglionic synapses in the ganglia of the cardiac plexus after upper thoracic and cervical sympathectomy.* José F. Nonidez, Department of Anatomy, Cornell University Medical College.

Examples of pericellular baskets (preganglionic synapses) in the cardiac ganglia of a cat 27 days after bilateral removal of the upper thoracic sympathetic chains and 60 days after extirpation of the cervical sympathetic. The effect of sympathectomy is not noticeable in these ganglia. This shows clearly that the neurones in the ganglia of the cardiac plexuses are in synaptic relation with preganglionics conveyed by the vagus nerve, i.e., they are parasympathetic. No sympathetic ganglia occur in these plexuses. Lack of continuity of the finer branches of the baskets with the neurofibrils of the neurones is also evident since the latter are faintly stained. A few terminal rings are visible in the preparations. The technique used was the chloral hydrate formula of the method of Cajal.

D49. *Transplantation of the biceps tendon through the head of the humerus. (Nicola operation.)* W. I. Norton and O. A. Mortensen, Department of Anatomy, University of Wisconsin.

The tendon of the long head was divided at its fleshy end and a drill hole placed through the upper end of the humerus from the intertubercular sulcus to the center of the articular surface. The tendon was threaded through this channel, reunited, and sutured to the periosteum. The animals (dogs and rabbits) were examined after periods of 4 days to 9 months. In all instances the tendon had become firmly fixed in the channel, the portion between the scapula and the humerus simulated the ligamentum teres. In some cases this portion was frayed, most often it was normal.

Histological examination showed no union of the tendon to the articular cartilage but firm connection to the walls of the bony channel. This union was effected by early organization of the clot and the proliferation of connective tissue from the bony wall. After 9 months the channel is filled with highly vascularized connective tissue, fat, and bundles of the transplanted tendon. Many of the larger blood vessels show endarteritic changes. The bundles of the transplanted tendon are obscure; they are widely separated by the newly formed connective tissue, and are atrophic.

The transplanted tendon has been incorporated into and in part replaced by the tissue which repairs the bony defect. Our material probably does not represent the final picture.

D50. Transthalamic connections of the pulvinar. James W. Papez, Department of Anatomy, Cornell University, Ithaca.

Removal of the suprasylvian gyrus in the cat produced severe degeneration of cortical efferent fibers to the pulvinar and nucleus lateralis. Some ended in the tectum.

In three human brains, lesions of the posterior parietal cortex were found associated with degenerations of the pulvinar and lateral nucleus. Demyelination of the tectum was present. In one case the pulvinar was severely atrophic. There was an extensive loss of fibers between the pulvinar and posterior and lateral sides of the medial nucleus. The anterior disseminate nucleus of the thalamus which formed the lobulated lateral part of the medial nucleus was atrophic and devoid of neuropil.

These observations led to study of these fiber connections in other human series and in series of the brains of monkey, dog and horse. In all series an extensive transthalamic system of fibers was traced from the pulvinar and nucleus lateralis into the posterior and lateral border of the medial nucleus. This region was composed of clusters of neurons surrounded by neuropil, forming a disseminate nucleus. This and the medial nucleus were connected to the frontal cortex through the anterior thalamic radiation.

The results suggest an extensive transthalamic connection between the posterior association area and the frontal cortex, and back again.

D51. Animated motion pictures as a method for illustrating anatomy. Anthony A. Pearson, Department of Anatomy, Loyola University School of Medicine, Chicago.

Animated motion pictures can be made from the serial sections of a brain or an embryo, either from drawings or from photographs of the sections. This method will be illustrated by a simple motion picture made from the drawings of each section of the brain of a human embryo. Every section was projected with reference to the outline drawing of the preceding section and traced with India ink. To insure proper registration, a peg board was used both in making and in photographing the tracings. Instead of the usual one or two frames, four were taken of each drawing so that the changes in configurations from section to section would not occur too rapidly. The 344 drawings can be shown in about 90 seconds. Several runs of the animation are included as only a few structures can be followed at once. Two models are included for the purpose of orientation. This film is submitted not so much for its own intrinsic value, but rather as a method of presentation which may be further developed.

D52. Sections showing the nerve fibers in the adult human hypophysis. A. T. Rasmussen, Department of Anatomy, University of Minnesota. (See abstract no. 141.)

D53. The terminal web in mammalian epithelia. Mary Elmore Sauer, Department of Histology and Embryology, University of Texas. (Introduced by Donald Duncan.)

A membrane of interlacing fibrils over the distal end of columnar epithelial cells at the level of the terminal bars was described by F. C. Sauer in the epithelia of a representative group of vertebrate embryos. De Rényi has found a similar structure in the snake. Examination of a wide variety of adult mammalian epithelia has shown the terminal web to be of common occurrence here also. It is frequently of a diffuse type, which is not closely confined to one plane and is correlated with double or multiple terminal bars. At least some of the fibrils appear to be continuous from cell to cell. Slides are presented of the salivary ducts of the adult rat, showing the terminal web in both tangential and transverse sections.

D54. Neurosecretory cells in vertebrates. Ernst Scharrer, Department of Anatomy, The University of Chicago. (Introduced by C. Judson Herrick.)

Neurosecretory cells are shown as they are found in sections of brains of teleosts, amphibia and mammals. By the term 'neurosecretory cell' is meant nerve cells having more or less the appearance of secretory cells. They may form well-delimited 'organs' within the central nervous system, as, for example, the diencephalic gland of vertebrates. Neurosecretory cells have a wide distribution among invertebrates and vertebrates including man. They are either true nerve cells or are derivatives of nerve cells. The neurosecretory cells produce granules and drops of colloid the staining properties of which are non-specific. It is as yet unknown what substances are secreted by glandular regions of the central nervous system and what is their physiological role.

D55. The growth and calcification patterns of the human deciduous teeth. Isaac Schour, Department of Histology, College of Dentistry, University of Illinois. (See abstract no. 152.)

D56. A study of the argyrophil fibers of the mouse thymus. Christianna Smith, Department of Zoology, Mount Holyoke College. (See abstract no. 157.)

D57. The response of the internal ear of the albino rat to intra vitam injection of trypan blue. Benjamin Spector, Department of Anatomy, Tufts College Medical School.

The object of this investigation is the study of the reaction of the periotic spaces, its associated structures and the organ of Corti to the intraperitoneal injection of trypan blue. Ten Wistar rats ranging in age from 12 days to 130 days were given intraperitoneal injections of 1 cc. of a 1% solution. The microscopic examination of these tissues shows that the trypan blue was transported from the blood stream chiefly by monocytes which phagocytosed the dye in the form of dust-like particles, small discrete granules or as larger masses. The connective tissues in the region of the secondary tympanic membrane and the

steps shows many monocytes heavily laden with trypan blue. The mesenchymal cells in the scalae, especially those in relation to the basilar membrane, take up this dye. Occasional cells containing trypan blue granules may be seen in the cells of the organ of Corti and in the tectorial membrane.

D58. Reactions of skeletal and cardiac muscle fibers to injury with special reference to the transformation of cross-striated substance into retraction clots.

Carl Caskey Speidel, School of Anatomy, University of Virginia.

These ciné-photomicrographs show the formation of retraction caps and clots in amphibian (frog), mammalian (rat), and arthropod (crab) skeletal and cardiac muscle fibers, as these are subjected to injury. The following points are illustrated: the susceptibility of the muscle tendon junction to early electrical injury, the formation of thick hyalin clots of skeletal muscle (Zenker's degeneration), recovery within a few minutes after thin retraction cap formation, fibers fragmenting at the junction of retraction cap and tendon and at the junction of retraction cap and retracting muscle, the torn ends of myofibrils, swollen myofibrils within retraction caps viewed obliquely from one end, slow transformation of resting cross striae into contraction bands, slow transformation of single sarcomeres into discrete clots, hard clots formed in fibers after heat injury as seen in living tadpoles, fibers with greatly swollen cross striae, retraction clot at the middle of a vacuolated fiber, fibrillation and Q-disc granulation, persistence of fine cross striae in some retraction clots, cross striae of skeletal and cardiac fibers in rigor mortis, cardiac muscle fibers showing retraction clots, contraction bands, intercalated discs, coarse Z membranes with Q discs present or absent, thin clots at Z membranes following a series of single electric shocks, retraction clots in beating tadpole hearts, and intercalated discs in twitching cardiac fibers of the rat heart. (See also abstract no. 159.)

D59. The effect of testosterone-propionate on the X-zone of the mouse adrenal.

William F. Starkey and Edward C. H. Schmidt, Jr., Department of Biology, University of Pittsburgh, School of Medicine. (Introduced by John C. Donaldson.) (No abstract.)

D60. Senile changes in the human semilunar ganglion. R. C. Truex, Department of Anatomy, University of Minnesota. (Introduced by A. T. Rasmussen.)

(See abstract no. 174.)

D61. Sperm morphology in the ram. Virgene Warbritton, University of Missouri and U. S. Department of Agriculture, cooperating.

The appearance of the spermatozoa of the ram is largely determined during their development in the testis, but it may be modified by their subsequent environments. The nuclear and cytoplasmic development in the seminiferous tubules does not diverge from the course described in most other species until late in spermiogenesis when the nucleus becomes so much flattened that its staining is difficult and the acrosome is relatively minute. The acrosome is involved in the

attachment of the spermatid to the Sertoli cell and is frequently lost when the spermatozoon becomes free-swimming. This free-swimming spermatozoon in the tubule has a large middle piece which is greatly reduced in size by the time the sperm is ejaculated. The nucleus of the free-swimming spermatozoon is stained equally well by acid or basic dyes, but in surface view either of the dyes appears diffuse.

Defective or abnormal sperm may be found in the testis, in the semen, and in the female tract. Most of the head defects and some of the tail defects have their origin in the testis. Most of the tail defects arise after the sperm have left the testes either in the epididymis, in stored semen, or in the female tract. Destruction of the tails is more rapid in the female tract than in other places, and is most rapid in the fallopian tubes of any place in the tract.

D62. Interrelationships of the hormones of reproduction in the human female as summarized by a mechanical demonstrator. Wayne L. Whitaker and R. Dean Schick, Departments of Anatomy and of Zoology, University of Michigan. (Introduced by Bradley M. Patten.)

The effects and interrelationships of the various hormones involved in the reproductive cycles of the human female present an exceedingly complex picture. This picture is particularly difficult to comprehend because of the ever-changing succession of conditions. It has seemed worthwhile, therefore, to attempt the construction of an apparatus which will show graphically, by changing levels of colored liquids in glass tubes, the relative amounts of the various hormones present at any given time during the menstrual or pregnancy cycles. Some effects of these hormones on certain organs of the body are indicated by a chart moving synchronously with the rise and fall of the liquids. During the menstrual cycle these distinctively colored liquids in separate tubes appear, symbolizing the follicle-stimulating factor of the pituitary, folliculin, luteinizing hormone, and progesterin. Soon after the fall of folliculin and progesterin the end of the cycle is indicated. A new one follows automatically. If, however, a special lever is actuated at any time during the assumed fertile period, menstruation does not occur, and the story of pregnancy is substituted. The anterior pituitary-like hormone is shown in a rapid rise and the estrin of pregnancy increases gradually. After delivery both disappear. Then prolactin rises rapidly and is continued for a time, after which the machine automatically reverts to the menstrual cycle.

D63. Living autogenous grafts of thyroid follicles as seen microscopically in a transparent chamber installed in the rabbit's ear. Roy G. Williams, Department of Anatomy, University of Pennsylvania. (No abstract.)

D64. Microscopical preparations of the pancreas of the guinea pig, after continuous intravenous injection of dextrose. C. Arthur Woerner, Department of Anatomy, The University of Chicago. (Introduced by R. R. Bensley.) (See abstract no. 182.)

D65. *A note on the distribution of nerves to the small blood vessels in the mesentery of the frog.* Benjamin W. Zweifach, Department of Anatomy, Tufts College Medical School. (Introduced by Benjamin Spector.)

Whole mounts of the mesentery of the frog were prepared by various silver and gold impregnation techniques. Unmyelinated nerves enter the mesentery along the larger blood vessels and subdivide into a number of wavy, smaller trunks which radiate throughout the tissue and anastomose with one another. Smaller branches of these further subdivide to form a diffuse plexus of fine, beaded nerve strands. The network is irregularly distributed and is joined by collaterals from the adventitial plexus found on nearby arteries and veins. Delicate ramifications of the nerve network parallel and lie in close proximity to capillary vessels and their branches.

Arterioles usually have two nerves accompanying them. One is in close contact with the vessel wall but fades out as the vessel approaches its venous termination. A second nerve spirals about the vessel and receives twigs from adjacent nerves in the general tissue network. A-v vessels are closely entwined by delicate nerves originating in the diffuse tissue plexus. The true capillaries are less regularly followed by nerve fibers, but few vessels are completely devoid of them. No dense pericapillary nerve plexus could be seen.

Two types of nerve termination occasionally could be found: 1) delicate beaded fibers seem to end on or fuse with the capillary endothelium; 2) branches of the nerve network terminate in large cells irregularly located throughout the tissue.

NEW BOOKS AND PUBLICATIONS

SYNTHETISCHE MORPHOLOGIE DER NIERE DES MENSCHEN, BAU UND ENTWICKLUNG DARGESTELLT AUF NEUER GRUNDLAGE, von Prof. Dr. Martin Heidenhain, Tübingen. 1937. Size, $7 \times 10\frac{1}{4}$ inches. Pages xvi + 270. 88 illustrations, 5 of which are in color. Price, 10.00 Guilders, E. J. Brill, Leiden.

LE THORAX ANATOMIE MEDICO-CHIRURGICALE, by Pr. Hovelacque, Olivier Monod and Henri Evrard. 1937. Size, $7\frac{1}{2} \times 10\frac{3}{4}$ inches. 356 pages. 125 illustrations. Index. Price, 150 francs. Librairie Maloine, Paris.

LIFE AS A WHOLE, by J. W. Bews, M.A., D.Sc., Principal of Natal University College. 1937. Size, $5\frac{1}{4} \times 8\frac{1}{4}$ inches. Pages ix + 347. Bibliography. Index. Cloth. Price, \$6.00. Longmans, Green and Company, New York.

“In this work ecological synthetic methods are applied in such a way as to obtain an all-round view of the meaning of life as it appears in the light of modern scientific research. The methods of investigating natural wholes are described and a sketch is given of the story of the evolution of living wholes in Nature. The different stages of man's life history are dealt with and his own efforts in creating new wholes—his inventions, institutions, philosophical ideas and the different branches of his art.” The publishers.

TRAILER AHOY! by Charles Edgar Nash. Being as comprehensive a book on the automobile house trailer as is possible for one man to prepare at this time from his own experiences and the fragmentary data available in an industry frantic with the demands of production. 1937. Size, $6 \times 8\frac{1}{4}$ inches. Many photographic illustrations. Fabrikoid. The Intelligencer Printing Company, Lancaster, Pa.

“That all America may realize the position the automobile house trailer is making for itself in our changing scheme of national life and that the advantages, short-comings and opportunities it presents may be thoroughly appreciated, this book has been produced.” The Publishers.

FEARFULLY AND WONDERFULLY MADE, The Human Organism in the Light of Modern Science, by Renée von Eulenburg-Wiener. 1938. Size, $6\frac{1}{2} \times 9\frac{1}{2}$ inches. Pages xiv + 472. 18 illustrations. Index. Bibliography. Buckram. Price, \$3.50. The Macmillan Company.

This book deals with the functions of the human organism, its physiology, anatomy and behavior as a whole. Life is an energy phenomenon and the functions of the body, including its sense perceptions and mental processes, are interpreted in terms of the energy concepts of modern physics. Describing the organs and the

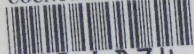
NEW BOOKS AND PUBLICATIONS

various functions and investigating the physico-chemical laws applicable to them, the author, time and again, is able to point to the asymmetry of the molecule as the key to the phenomena of life. Scientists have known about this characteristic property of the bio-molecule, its asymmetry, for almost 100 years, and to the great Pasteur it was the one distinct line of demarcation between the organic and the inorganic world. Yet, the important implications of the peculiar groupings of the atoms in the bio-molecules had to await the advent of the new physics in order to be fully appreciated. The significance of molecular asymmetry runs like a guiding thread through this book. Within astonishingly short compass the author has succeeded in presenting and explaining the most important facts now known about the human body. This book should be of interest not only to the student, but to the layman who wishes to know more about the human organism. The Publishers.

PAVLOV AND HIS SCHOOL. The Theory of Conditioned Reflexes, by Prof. Y. P. Frolov, M.D., Member of the All Union Institute of Experimental Medicine, Moscow. Size, $5\frac{1}{4} \times 8\frac{1}{4}$ inches. Pages xix + 291. 26 illustrations. Index. Cloth. Price, \$4.00. Oxford University Press, New York.

The author of this book has been one of Pavlov's pupils and fellow-workers. He has written an intensely personal document containing biographical information about "the surging cataract of energy which we know by the name of Pavlov" as well as an account of his work. The chapter titles give the scope of the work. Pavlov's Immediate Predecessors: Sechenov and the Behaviorists. Pavlov's Theory of Conditioned and Unconditioned Reflexes. The Analysis of Complicated Forms of Behavior from the Standpoint of Conditioned Reflexes. Pavlov's Views on the Inhibition of Conditioned Reflexes. Pavlov's Theory of Sleep and States Akin to Sleep. Experiments with Anthropoid Apes, and the Comparative Physiology of Conditioned Reflexes. Pavlov's Theory of Experimental Neuroses. Pavlov's Method of Work: his School. It is a clear and concise account, demanding no extensive knowledge of nervous physiology, and showing by numerous examples from daily life the application of Pavlov's teaching to subjects not generally associated with physiology.

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